Amit Rastogi et al. 2017, Volume 5 Issue 3
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

International Journal of Science, Engineering and Technology
An Open Access Journal

Optimization of Media for Laccase Production Using Pseudomonas Mendocina

[1]Amit Rastogi, [2]Poonam Singh

Introduction

In the recent years, enzymes have gained great importance in Industries; laccases are one among them which are widely present in the nature. Laccases are the oldest and most studied enzymatic systems [1].

Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) is a part of broad group of enzymes called polyphenol oxidases containing copper atoms in the catalytic center and are usually called multicopper oxidases. Laccases contain three types of copper atoms, one of which is responsible for their characteristic blue color. The enzymes lacking a blue copper atom are called yellow or white laccases. Typically laccase-mediated catalysis occurs with reduction of oxygen to water accompanied by the oxidation of substrate. Laccases are thus oxidases that oxidize polyphenols, methoxy-substituted phenols, aromatic diamines, and a range of other compounds [2].

Laccases are widely distributed in higher plants, bacteria, fungi, and insects. In plants, laccases are found in cabbages, turnip, potatoes, pears, apples, and other vegetables. They have been isolated from Ascomyceteous, Deuteromycteous and Basidiomycetous fungi to which more than 60 fungal strains belong [3]. The white-rot Basidiomycetes fungi efficiently degrade the lignin in comparison to Ascomycetes and Deuteromycetes which oxidize phenolic compounds to give phenoxy radicals and quinines [5].

Laccases play an important role in food industry, paper and pulp industry, textile industry, synthetic chemistry, cosmetics, soil bioremediation and biodegradation of environmental phenolic pollutant and removal of endocrine disruptors [6]. These enzymes are used for pulp delignification, pesticide or insecticide degradation, organic synthesis [4], waste detoxification, textile dye transformation, food technological uses, and biosensor and analytical applications.

Recently laccases have been efficiently applied to nanobiotechnology due to their ability to catalyze electron transfer reactions without additional cofactor. The technique for the immobilization of biomolecule such as layer-by-layer, micropatterning, and self-assembled monolayer technique can be used for preserving the enzymatic activity of laccases.

Method and materials

Media for laccase production

Media was composed of **NaH_2PO_4 800 mg, KCl 200 mg, $MgSO_4$. $7H_2O$ 500 mg, NH_4NO_3 200 mg, Yeast Extract 10 gm, $CaCl_2$ 10mg, $CuSO_4$. $5H_2O$ 500 mg, $FeSo_4$. $7H_2O$ 5mg, $MnSO_4$ 5 mg, Glucose 1gm.** per 1 litre media.

Enzyme Assay

Laccase assay was performed using syringaldazine as substrate. Reaction mixture was prepared using 3 ml of Phosphate buffer (100 mM, pH-7), 1ml of 1mM syringaldazine (in absolute ethanol) and 1ml of cultural filtrate where as in blank 1ml of distilled water was used instead of cultural filtrate.

Reagents	Blank	Test
100mM Phosphate Buffer pH- 7	3 ml	3ml
1mM Syringaldazine (in absolute ethanol)	1ml	1ml
Cultural Filtrate	1ml Distilled water	1ml

The change in absorbance due to the oxidation of syringaldazine (ε=65,000 M−1 cm−1) in the reaction mixture was monitored for 10 min of incubation at

530nm and 30^0C. Laccase activity was expressed in U/ml. U/ml was defined as production of 1uM of colored product/min/ml.

Optimization of Carbon source for Laccase production

For this purpose lactose, maltose, sucrose, starch and galactose were utilised to optimised carbon sources in the production medium.

Optimization of Nitrogen source for Laccase production

Different nitrogen sources such as peptone, beef extract, NH4Cl, NH4SO4 and urea were used for laccase production for Pseudomonas mendocina at 37^0C after 72 hours incubation.

Optimization of pH for Laccase production

The influence of pH on the production of laccase was studied at different pH range of 5 to 11.

Optimization of Time duration for Laccase Production

The effect of time duration on laccase production using Pseudomonas mendocina was observed for 192 hours from 24 hours in laccase production media at 37^0C and pH 7.0 in 250 ml Erlenmeyer flask.

Optimization of Temperature for Laccase Production

The temperature was optimized for the better production of laccase. It has profound influence on production of laccase by microorganisms. In order to determine the enzyme production, cells were incubated at 0^0C, 10^0C, 20^0C, 28^0C, 37^0C and 50^0C and the growth of the Pseudomonas mendocina was observed.

Result

Optimization of Carbon source for Laccase production

The uses of cheap carbon sources are important as these can significantly reduce the cost of production of laccase. Therefore, the utilization of various carbon sources such as lactose, maltose, sucrose, starch and galactose were evaluated at 37^0C after 72 hours incubation and agitation with respect to enzyme yield. The best carbon source for AR15 was sucrose as its produce maximum enzyme 0.169 U/ml at 37^0C after 72 hours incubation, which was closely followed by Maltose with enzyme activity 0.0866 U/ml and starch with enzyme activity 0.083U/ml for AR15 at 37^0C after 72 hours incubation.

Table: Optimization of carbon source

Carbon sources	Enzyme activity U/ml
Lactose	0.0816
Maltose	0.0866
Sucrose	0.169
Starch	0.083
Galactose	0.028

Optimization of Nitrogen source for Laccase production

Different nitrogen sources such as peptone, beef extract, NH4Cl, NH4SO4 and urea were used for laccase production for AR15 at 37^0C after 72 hours incubation. Beef extract was found as best nitrogen source for AR15 with enzyme activity 0.206U/ml at 37^0C after 72 hours incubation which was closely followed by peptone with enzyme activity 0.199U/ml and ammonium chloride with enzyme activity 0.156U/ml at 37^0C after 72 hours incubation where as minimum enzyme activity (0.095U/ml) for AR15 was found using ammonium sulphate as nitrogen source at 37^0C after 72 hours incubation. [7,8]reported maximum enzyme production was achieved when using peptone[9] reported laccase was also produced when the fungus was cultivated in nitrogen rich media rather than nitrogen-limited media.

Table Optimization of Nitrogen source

Nitrogen Sources	Enzyme activity U/ml
Peptone	0.199
Beef	0.206
NH4Cl	0.156
NH4SO4	0.095
Urea	0.171

Optimization of pH for Laccase production

The pH of culture strongly affects many enzymatic processes and transport of compounds across the cell membrane. The influence of pH on the production of laccase was studied at different pH range of 5 to 11. The isolate AR15 produced maximum laccase at pH 7.0 (0.189U/ml), after 72 hours incubation at 37^0C. The enzyme production from AR15 was decreases with increase in pH except pH 10 and produced 0.172, 0.128, 0.168 and 0.128U/ml enzyme at pH 8,9,10 and 11, respectively. However, at pH 5 and 6 only 0.105 and 0.125U/ml enzyme was produced. The optimum pH-7 for laccase production by using *Chaetomium globosum* was reported by [7].

Table Optimization of pH

pH	Enzyme activity U/ml
pH- 5	0.105
pH- 6	0.125
pH- 7	0.189
pH- 8	0.172
pH- 9	0.138
pH- 10	0.168
pH- 11	0.128

Optimization of Time duration for Laccase Production

The effect of time duration on laccase production using AR15 was observed for 192 hours in laccase production media at 37^0C and pH 7.0 in 250 ml Erlenmeyer flask. AR 15 started the production of laccase from 24 hours of growth and reached maximum in 120 hours (0.135U/ml). At 144, 168 and 192 hours 0.133, 0.069 and 0.047 U/ml of enzyme was produced. The enzyme production was gradually increases with incubation time up to 120 hours and it was found that at 24, 48, 72 and 96 hours of incubation 0.087, 0.091, 0.106 and 0.116 U/ml enzyme was produced, respectively. The maximum enzyme production reached on 120 hours using *Chaetomium globosum* was reported by [7]. [8] also reported the maximum enzyme production on 120 hours by using newly isolate *Pleurotus ostreatus* LIG 19. [9] Also reported maximum laccase activity was achieved on 120 hours using *Trametes versicolor* IBL-04.

Table Optimization of time duration

Time Duration	Enzyme activityU/ml
24 Hours	0.087
48 Hours	0.091
72 hours	0.106
96 Hours	0.116
120 Hours	0.135
144 Hours	0.133
168 Hours	0.069
192 Hours	0.047

Optimization of Temperature for Laccase Production

The temperature was optimized for the better production of laccase. It has profound influence on production of laccase by microorganisms. In order to determine the enzyme production, cells were incubated at 0^0C, 10^0C, 20^0C, 28^0C, 37^0C and 50^0C and the growth of the AR15 was seen and it was ranked as: intense +++, moderate ++, slight+, and no growth -. It has been seen that temperature 37^0C showed maximum growth. So, at this temperature culture will show higher enzyme production. Same results were reported by [10].[11] reported 32^0C as optimum temperature for laccase production from *Streptomyces psammoticus*.

Table Optimization of temperature

Temperature	Growth observation	Growth Type
0^0C	-ve	No Growth
10^0C	-ve	No Growth
20^0C	+ve	Slight Growth
28^0C	++ve	Moderate Growth
37^0C	+++ve	Intense Growth
50^0C	-ve	No Growth

Discussion

On the behalf of result sucrose was found best carbon source where as best nitrogen source was Beef. Later on optimum pH was found pH-7 and 120hour was found best time duration. To determine best temperature microbial growth was seen on different temperature and 37^0C was found as optimum temperature.

References

[1]P. R. Williamson(1994) "Biochemical and molecular characterization of the diphenol oxidase of cryptococcus neoformans: identification as a laccase," Journal of

Bacteriology, vol. 176, no. 3, pp. 656–664. View at Google Scholar

[2] Baldrian P. Fungal laccases-occurrence and properties. FEMS Microbiology Reviews. 2006;30(2):215–242. [PubMed]

[3]L. Gianfreda, F. Xu, and J. M. Bollag(1999)"Laccases: a useful group of oxidoreductive enzymes," Bioremediation Journal, vol. 3, no. 1, pp. 1–25 View at Google Scholar

[4]J. Faccelo and O. Cruz(2008), Banana skin: a novel material for a low-cost production of laccase, M.S. thesis, Universitat Rovira I Virgili.

[5]C. Eggert, U. Temp, J. F. D. Dean, and K. E. L. Eriksson,(1996) "A fungal metabolite mediates degradation of non-phenolic lignin structures and synthetic lignin by laccase," FEBS Letters, vol. 391, no. 1-2, pp. 144–148. View at Publisher • View at Google Scholar

[6]S. R. Couto and J. L. Toca Herrera (2006), "Industrial and biotechnological applications of laccases: a review," Biotechnology Advances, vol. 24, no. 5, pp. 500–513. View at Publisher • View at Google Scholar • View at PubMed

[7]El zayat S-A (2008). Preliminary studies on laccase production by Chaetomium globosum an Endophytic fungus in Glinus lotoides. Am. Eurasian J. Agric. Environ. Sci. 3(1):86-90.

[8]Sivakami, V., Ramachandran, B., Srivathsan, J., Kesavaperumal, G., Benila, S. and Mukesh, K. (2012). Production and optimization of laccase and lignin peroxidase by newly isolated Pleurotus ostreatus LIG 19. Journal of. Microbiology and Biotechnology Research 2, 875-881.

[9]Heinzkill M, Bech L, Halkier T, Schneider P & Anke T (1998)Characterization of laccases and peroxidases from woodrotting fungi (family Coprinaceae). Appl Environ Microbiol 64: 1601–1606.

[9]M. Asgher, H.M.N. Iqbal, and M.J. Asad (2012), Kinetic characterization of purified laccase produced from Trametes versicolor IBL-04 in solid state bioprocessing of corncobs, Bio Resources 7(1), 1171-1188.

[10]Singh G, Bhalla A, Kaur P, Capalash N, Sharma P. Laccase from prokaryotes: a new source for an old enzyme. Rev Environ Sci Biotechnol. 2011;10(4):309–326. doi: 10.1007/s11157-011-9257-4.

[11]Niladevi KN, Sukumaran RK, Jacob N, Anisha GS, Prema P. Optimization of laccase production from a novel strain-Streptomyces psammoticus using response surface methodology. Microbiol Res. 164: 105-113, 2009

Author's Details

[1]Department of Molecular and Cellular Engineering, Sam Higginbottom University of Agricultural, Technology and Sciences, Allahabad, India

[2]Department of Molecular and Cellular Engineering, Sam Higginbottom University of Agricultural, Technology and Sciences, Allahabad, India

Mail id : amitrastogi1986@gmail.com