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Research Article

Partial Purification and Characterisation of Laccase enzyme from B_{14}

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Abstract

In the current study bacterial strain was isolated from the various soil sample from the different soil samples from different region. Total 55 different bacterial and fungal isolates were isolated from the various regions of Surat. They were screened for Laccase production in BHM. Laccase activity was then determined using syringaldazine as a substrate. Isolate B_{14} was selected of a maximum laccase activity 302 U/ml. Effect of various parameter were studied such as pH, temperature, Carbon source, Nitrogen source, various metals, various concentration of syringealdazine. Further partial purification of enzyme was carried out using Ammonium Sulphate precipitation and the results were obtained 38.54% partially purified enzyme.

INTRODUCTION

Laccase is a part of broad group of enzyme, usually it's called as Multicopper oxidases and is found to be oldest and most studied enzyme. It's the organic substance available around the globe and is found to be more valuable biomass as it can be converted to various energy products(Desai Sagar A, 2017). Laccase are extracellular enzyme and over multi-copper enzyme that uses oxygen to carry out oxidation of various aromatic and non aromatic compounds by a radical reaction mechanism. (Vaidyanathan *et al*., 2011).

Laccase accommodates 3 types ofcopper atom of which is responsible for the characteristics of blue colour so also called as "Blue- Laccase" (Desai Sagar A, 2017). Enzymes lacking blue colour are called yellow or white laccase. They are widely distributed in the higher plants, fungus, and bacteria.

Even though laccase have wide occurrence in plants, Laccase is not been used and characterise because of their detection and purification process is found to be difficult. (Ranocha *et al.*, 1999). Laccase production can be stimulated by the presence of various .inducers such as aromatic or phenolic compounds.

Laccase plays an important role in different approach such as food industry, textile industry,and otherindustries etc. Recently laccase has been taken into study for nanobiotechnology because oftheir ability to catalyse electron transfer reaction without additional co-factor. The technique for immobilisation of molecule such as layer by layer, micro-patterning and self assemble mono-layer technique can be used for preserving the enzymatic activity of Laccase (Shraddha *et al*., 2011).

Aim of the present study was isolation and characterisation of bacterial isolate from agricultural land soil capable for the laccase production and establish the various physiological parameter for laccase production and further partial purification of the enzyme was carried out.

MATERIALS AND METHODS

Collection of sample:

Soil samples were collected from different regions of Surat and nearby region which have temperature range about 27 to 30ºC for isolation of Laccase producing bacteria. Here the isolate was isolated from the Bapalal Garden at V.N.S.G.U.

Isolation and Screening of Laccase producing bacteria:

For isolation of laccase producing bacteria 1g of soil was dissolve in 100ml of sterile BHM and then incubated at 37 ºC for 24 to 48 hours.

After incubation the plates were observed for various isolated colonies and was studied for morphological difference of bacterial isolate

Primary screening for laccase production was carried out by plating them on BHM agar plate with the addition of substrate syringaladazine (0.01%).

Development of dark brown colony on the medium indicated the production of laccase. The isolate which showed brown colour colonies within 12 hour of incubation was B14 which was further studied for secondary screening laccase enzyme production in BH broth supplemented with syringaldazine and checked for the enzyme activity for successive days and it was kept on rotator shaker at 150 rpm.

Enzyme Assay

Laccase activity was determined by Syringaldazine assay in which oxidation of syringaldazine at 530 nm. The reaction mixture (3 ml) which contained 0.1 ml of the enzyme sample and 2.9 ml of 20M syringaldazine in 50 mM sodium phosphate buffer, pH 7.0 was incubated at 30°C for 10 min. Enzyme activity was expressed in enzyme units. 1 U being defined as the amount of enzyme causing the formation of 1mol of product per minute under the assay conditions used. Protein assay was determined by Folin Lowry Estimation.

Laccase enzyme production using the Submerged method:

Laccase production was carried out using submerge fermentation method. Isolate obtained from the 2^{o} screening was selected for the further study of enzyme production

Effect of different carbon sources:

Different carbon sources were used for the study of effect on the laccase enzyme activity i.e. Glucose, Lactose, Sucrose at 0.5% W/V concentration.

Isolate B14 was inoculated in the basal medium in different Erlenmeyer flask and where incubated at 30ºC for 24 hours.Later the enzyme activity was measured for the 3 successive days.

Effect of Nitrogen sources:

Different nitrogen sources were used for the study of effect on laccase enzyme activity i.e. Peptone, Beef extract, Yeast extract.Isolate B14 was then inoculated in the basal medium in different Erlenmeyer flask at concentration of 0.5%.Later the enzyme activity measurement was carried out for the 7 successive days.

Effect of various metals:

There were different metals taken into present study to observe the effect of various metal ion concentration on the laccase enzyme activity. Metals studie were $CuSO_4$, $ZnSO_4$, $CdCl_2$. Isolate B14 were inoculated in the basal medium in different Erlenmeyer flask at 0.5% W/V concentration and each flask was incubated at 30ºC for 24 hours. Enzyme activity was carried out at for 7 successive days.

Partial purification of Laccase enzyme:

150 ml of sterile production medium which contains the composition of characterise medium was inoculated with 24 hours old culture of B14 and was incubated at 30ºC for 24-48 hours. After incubation the crude laccase was collected by centrifugation at 6000 rpm for 15 minutes. Supernatant was collected and preceded for the laccase activity and protein estimation.50ml supernatant were 1st precipitated by 30% saturation with ammonium sulphate. Precipitated protein was separated by centrifugation for 15 minutes at 6000 rpm. Pellet was dissolve in minimum volume of 100mM of Phosphate buffer. From 30% supernatant 80% of ammonium sulphate was proceeded and from the pellet30% and 80%activity was been measured.

RESULTS AND DISCUSSION

In the current study soil sample was collected from Bapalal garden V.N.S.G.U Surat. Enrichment of the sample was done to enhance the growth of bacterial population in the medium. For the sake of isolation enriched sample were streaked on Bushnell hass agar plate and the plates were incubated at 37ºC for 24 hours to 48 hours. There were 55 different isolates were isolated and screened for Laccase production in BHM + Syringaldazine as a substrate. Out of 55 isolates 20 isolates showed dark brown colonies. Out of these 20 isolates B_{14} was selected on the base of Laccase enzyme activity measurement. Selected bacterial isolate B_{14} was studied for the potential to produce Laccase enzyme under submerge condition. Maximum laccase activity observed at the end of 3 days is 292 U/ml.

Optimum pH of the laccase is dependent on the substrate used. In general laccase has been 'Bell' shape profile with an optimum pH that varies. Laccase which is produced by *Trametes modesta* is found to be active at pH 4.0 and is stable at pH 4.5 and half life decreases to 125 minutes at pH-3 (Kunamneni et al., 2007). When phenol is used as a substrate the optimum pH would be in the range between 3 to 7 for fungal laccase and pH-9 for plant laccase. While here B14 isolate was found to give highest activity at pH 6.5 and it was found that the laccase activity was found to be better in acidic condition using Syringaldazine as a substrate. When ABTS was used as a substrate the optimum pH was found to be acidic between 3 to 5. The result can vary because of the change in the reaction occurs because of substrate or enzyme. (Intwala Siddhi M., 2015)

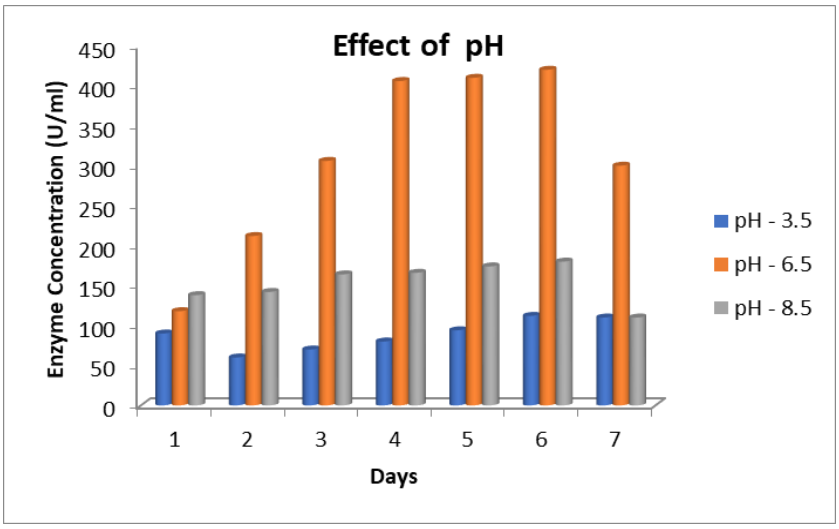

Figure:1 Effect of pH on Laccase activity

Optimal temperature of Laccase can be varied from one strain to another strain. Based on the literature review study of laccase using *Ganoderma lucidium* was found to be 20-25ºC and when isolated from *Marasmus querocorpium* was found to be 60ºC. And the study of laccase using *Pleuorotus ostreatus* was found to be at the range between 40 to 60 ºC with highest activity at 50 ºC. (Madhvi et al 2009). Isolate B14 was studied for the effect on enzyme activity at 30 ºC, 40 ºC and 20 ºC. Maximum activity was observed at 30ºC and the temperature range will be varied from one species to another species. (Sagar Desai., 2017).

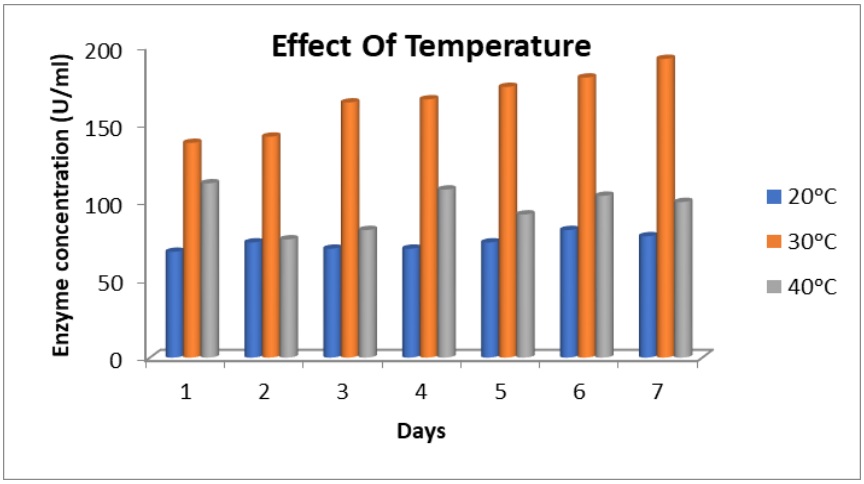

Figure:2 Effect of Temperature on Laccase activity

While GIDC P1 (*Enterobacter cloacae*) showed better activity at 50 ºC while our isolate B14 showed highest activity at 30 ºC so it can be said that the temperature ranges varies from one species to another species (Intwala, 2015).

Carbon plays and important role in the medium. Based on the literature review it was found that glucose and cellobiose were efficiently utilize by *Trametes pubescens* with higher laccase activity. (Kunamneni *et al*., 2007).

While *Enterobacter cloacae* isolate also showed the greater activity with glucose as a carbon source (Intwala Siddhi M, 2015). Here also 3 different carbon sources played an important role in enzyme production carbon sources which were utilized were glucose, sucrose and lactose. Here the B14 showed the maximum activity with the glucose as a carbon source. i.e. 318 U/ml. Utilization of the carbon sources may vary from one species to other species.

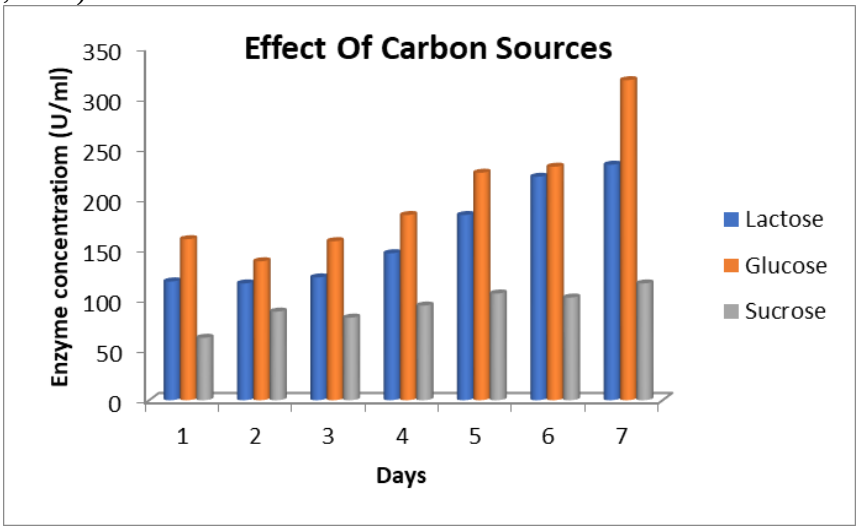

Figure:4 Effect of Carbon source on Laccase

Effect of the nitrogen source in the medium play an important role in enzyme production. Some studies shows that the elevation in laccase activity was achieved by low carbon to nitrogen ratio while other had higher carbon to nitrogen source. (Shraddha *et al*., 2011). Contradictory evidence on the effect of nitrogen source on laccase activity was also observed. High N media has shown the maximum production of laccase and some showed the better production in the nitrogen deficient medium. *Enterobacter cloacae* gave better enzyme activity with Peptone as a nitrogen source. Different N-source which can be utilized for the study of effect on laccase activity are Beef extract, yeast extract and peptone as a nitrogen source.

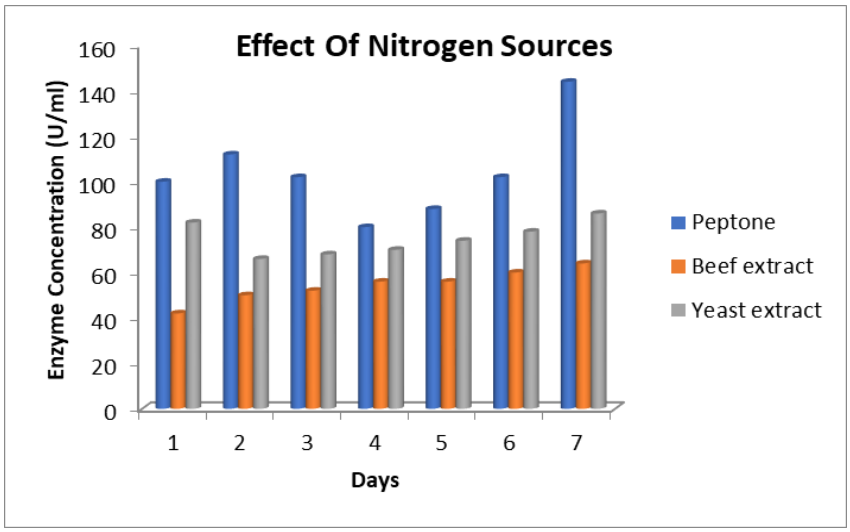

Figure:5 Effect of Nitrogen source on Laccase

Different metal ion concentration on enzyme activity showed quite variability result which showed the increase in enzyme activity using various metals $CdCl_2$, $ZnSO_4$, and $CuSO_4$ at different concentration. According to the literature review on the supplementation of $CuSO_4$ at different concentration enzyme activity was found to increase. (Desai Sagar A., 2017).

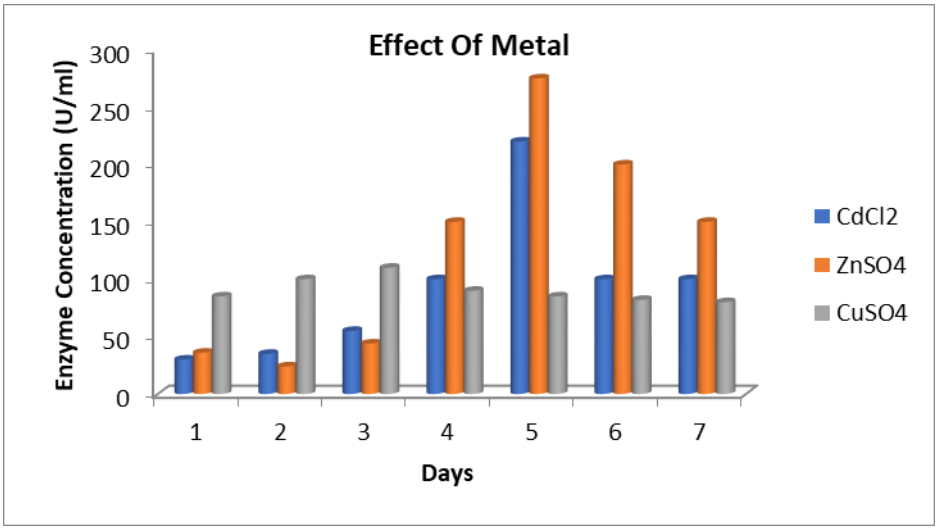

Figure:6 Effect of Metal on Laccase activity

By performing the laccase enzyme precipitation by using Ammonium Sulphate, from the result at 30% and 80% purification showed the 38.54% purity in the 30 ml broth. Which was quite good? And these partially purified enzymes can be further studied for the purification purposes.

Fraction	Volume	Enzyme activity U/ml	Total activity U/ml	Total protein Mg/ml	Specific activity Units	Fold purification U/ml	% of yield
Crude	50	1496	8.25	6.85	1.29	0.156	100
30%	40	1728	7.48	5.48	1.36	0.164	90.6
80%	30	1004	3.18	5.16	0.61	0.073	38.54

Figure:7 Partial purification of Laccase

Competing Authors:
Authors declare that they have no competing interests.

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REFERENCES

Alcalde M, Bulter T, and Arnold FH, 2014. Colorimetric Assays for Biodegradation of Polycyclic Aromatic Hydrocarbons by Fungal Laccases . *Journal of biomolecular screening,* **7**(6): 547-553.

Chawachart N, Khanongnuch C, Watanabe T and Lumyong S, 2004.

Desai SS, Tennali GB, Channur N, Anup AC, Deshpande G and Han MJ, Choi HT, and Song HG, 2005. Purification and Characterization of Laccase from the White Rot Fungus Trametes versicolor. *The Journal of Microbiology,* **43**(6): 555-560.

Intwala SM., Purohit MK., Desai PV. 2015. Partial Purification and Characterisation of Dye Degrading Enzyme Laccase from Enterobacter cloacae Strain. *International journal of sciences &Applied research,* **2**(6); 49-55.

Kunamneni A, Ballesteros A, Plou F and Alcalde M, 2007. FungalLaccase – A Versatile Enzyme For Biotechnological Application. Communicating current research and educational Topics and trends in applied microbiology. 233-245.

Madhavia V and Lele S, 2009. Laccase: properties and applications. *Bioresources*, **4**(4):1694-1717.

Murtuza A, 2011. Isolation of Laccase Producing Fungi and Partial Characterization of Laccase. *Biotechnology, bioinformatics Bioengineering*, **1**(4): 543-549.

Rice bran as an efficient substrate for laccase production from thermo-tolerant basidiomycete Coriolus versicolor strain RC3. *Fungal Diversity*, **15:** 23-32.

Sagar A Desai, 2017. Isolation and characterization of Laccase producing bacteria from contaminated sites. *Bioscience Discovery*, **8**(3):567-573.

Satpathy R, Behera R, Padhi S, Guru R, 2013. Computational Phylogenetic Study and Data Mining Approach to Laccase Enzyme Sequences. *Phylogenetics & evolutionary biology*. **1**(2): 1-7.

Shraddha, Shekher R, Sehgal S, Kamthania M, and Ajay Kumar, 2011. Laccase: Microbial Source, Production, Purification, and Potential Biotechnological Application. SAGE-Hindawi Access to Research.

Vaidyanathan V, Kirupha SD, Periyaraman P And Subramanian S, 2011. Screening and Induction of Laccase Activity in Fungal Species and its Application in Dye Decolorization. *African journal of Microbiology research*, **5**(11):1261-1267.

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