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PRODUCTION AND PURIFICATION STRATEGIES FOR LACCASE

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ABSTRACT: Laccase belongs to polyphenol oxidases, which is a very wide family of copper-containing atoms in the center, which serves as the catalytic site. It is biochemically a glycoprotein with molecular mass being between 50kda to 130kda and 30% carbohydrate content, in addition to this it has three types of copper atoms. It is one of the most important enzymes due to its role in the food industry. It acts as a biosensor where it can detect morphine, codeine, catecholamine, and many other enzymes. In insects, it performs the function of cuticle sclerotization. Moreover, laccase is safe for human consumption. The most important factor which has prevented laccase from being insanely famous is the cost that is associated with using a very large quantity of laccase. Laccases can be extracted from almost all kinds of white-rot fungi as well as from various other fungi species such as *Pycnoporus cinnabarinus, Gloeophyllum trabeum* but not in any brown-rot species except *Coniophora puteana* as was recently discovered. *Aspergillus niger, Aspergillus oryzae* and *Trichoderma reesei* also produce laccases due to the laccase producing genes introduced in them. *Botryo sphaeria* is a true laccase producing fungi, not only this, it can also be extracted from plants, bacteria such as *Escherichia coli, Bacillus halodurans* but not much work has been done on any other organism except fungi. Bacterial laccase is highly thermos-tolerant and functions in neutral to alkaline conditions, while fungal laccase, on the other hand, does not function at high temperature and pH.

INTRODUCTION: Enzymes play a major role in the metabolism of all living organisms. Extra-cellular enzymes have gained great importance in the industrial sector. Among those, laccase is one of the most used enzymes due to its applications in various fields.

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It is a type of copper-containing polyphenol oxidase that was discovered in the exudates of the Japanese lacquer tree *Rhus verniczfera* and it cross-links the monomers, degrades the polymers, and helps in ring cleavage of aromatic compounds [1].

Laccases are abundantly found in higher plants, bacteria, fungi as well as in some of the insects. The plant sources of it being *Malus pumila, Brassica oleracea, Brassica rapa, Solanum tuberosum, Pyrus calleryana* and some vegetables [1, 2]. Fungal laccases are considered as the hometown of laccase because more than 60 fungal strains like Ascomycetes, Deuteromycetes and

Basidiomycetes belonging to the white-rot basidiomycetes are the huge sources of laccase that can be isolated [3, 4]. These fungal laccases play an important role in the plant pathogenesis, pigment production, and degradation [5]. These enzymes are also used in the synthesis of organic substance [6]. Here the typical substrates are amines and phenols and the reaction products are dimers and oligomers which are derived from the coupling of reactive radical intermediates [1, 7].

In recent years, these enzymes have gained platform in the field of textile, pulp, paper and food industry and surprisingly it is also used in the design of biosensors, biofuel cells, as a medical diagnostics tool and acts as bioremediation agent which clean up herbicides, pesticides and also certain explosives in soil [2, 5, 7]. Laccases can oxidize both phenolic and non-phenolic lignin-related compounds as well as highly recalcitrant environmental pollutants, this made the researchers turn towards laccase from last decade [1, 5]. Laccases continue its contribution and play an important role in synthetic chemistry, cosmetics, soil bioremediation, pulp delignification, biodegradation of environmental phenolic pollutants and removal of endocrine disruptors [3, 8]. Recently, laccases have been efficiently applied to nano-biotechnology as they can catalyze electron transfer reactions without additional co-factor [9]. The question of preserving these enzymes may arrive, but the technique for the immobilization of biomolecules such as layer-by-layer, micropatterning, and self-assembled mono-layer technique for preserving the enzymatic activity of laccases answers us well [3, 4, 6].

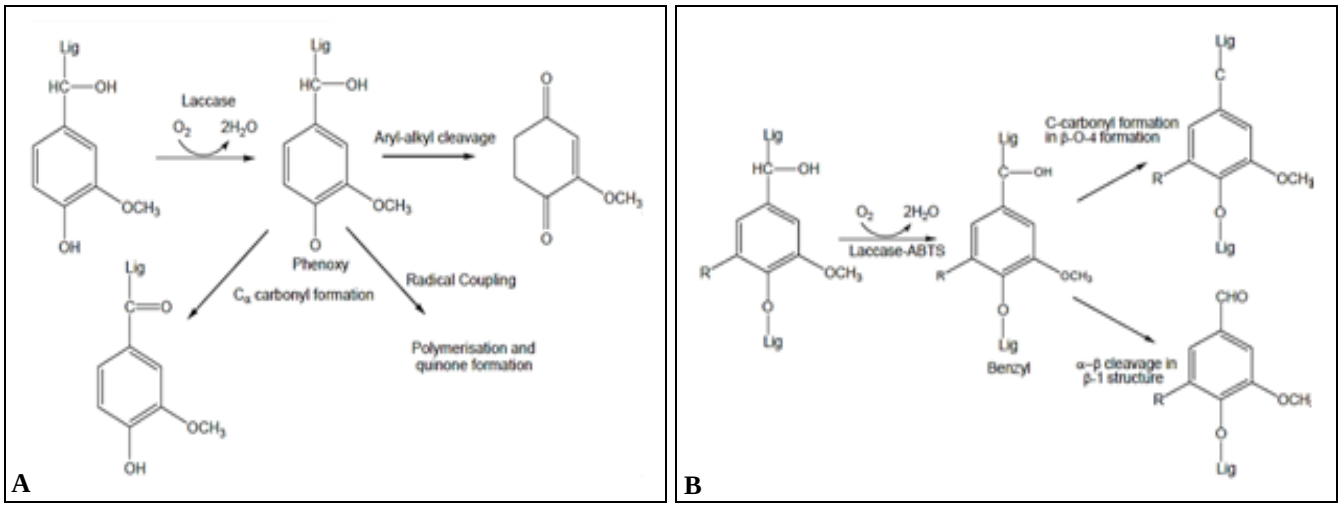

FIG. 1: OXIDATION OF (A) PHENOLIC SUBUNITS OF LIGNIN BY LACCASE AND (B) NON-PHENOLIC LIGNIN MODEL COMPOUNDS BY A LACCASE MEDIATOR SYSTEM

TABLE 1: USES OF LACCASE IN VARIOUS AREAS [1, 3, 6, 7, 10]

S. no.	Area	Application
1	Food Industry	1. Brewing 2. Colour enhancement in tea *etc*. 3. Cork modification 4. Juice processing 5. Wine stabilization 6. Provides stability as well as shelf life to beer
2	Paper Industry	1. Pulp bleaching 2. Paper pulp delignification
3	Textile Industry	1. Denim bleaching 2. Denim shading 3. Denim finishing 4. Dye decolorization
4	Agriculture	1. Bioremediation and biodegradation of pesticides and other harmful compounds 2. Bioremediation of wastewater

There are several production and purification strategies for laccase which are discussed below.

Sources: Laccase, one of the most important enzymes in the food sector, is on very high demand these days because of its industrial applications as well as health benefits; thus all its sources are extensively studied and researched upon. It can be extracted from higher plants, fungi and as recently discovered in *Bacillus subtilis*, *Bacillus halodurans*, *Azospirillum lipoferum*, *Saccharomyces lavendulae*, *Saccharomyces cyaneus*, and *Marinomonas mediterranea* bacteria, in case of higher plants laccase was found in *Sycamore maple*, *Rhus vernicifera*, *Mangifera indica*, *Schinusmolle*, *Pistachia palaestina* and

Pleiogynium timoriense [2, 7]. But the most important one is the fungi such as Basidiomycetes, white-rot fungi, many *Trichoderma* species such as *Trichoderma atroviride, Trichoderma harzianum,* and *Trichoderma longibrachiatum* are the sources of laccases. Laccase from the *Monocillium indicum* was the first laccase to be characterized by Ascomycetes which shows peroxidase activity.[6] In Basidiomycetes important fungi species are *Phanerochaete chrysosporium, Theiophora terrestris, Lenzites* and *betulina* while the important white-rot fungi being *Phlebia radiate*, *Pleurotus ostreatus,* and *Trametes versicolour* [1, 11].

Use of this Review: Various isolation, production and purification strategies were analyzed for further research on this enzyme.

Production: Enzymes are usually produced on a large scale using those strains of organisms that have the ability to overproduce it. The foremost step to produce any enzyme is to cultivate the organisms that produce the desired enzymes, it is then regulated and optimized under various fermentation conditions for overproduction of that enzyme. The two basic methods that are included under enzyme production are semi-solid culture and submerged culture. In the case of semi-solid culture. The enzyme producing culture is being grown on a surface of a suitable semi-solid substrate, whereas in case of submerged culture fermentation equipment is used that is a cylindrical tank of stainless steel, equipped with an agitator cooling system and various ancillary equipment (foam control, pH monitoring device, temperature, oxygen tension, *etc*.). There have been several factors that play a major role in the production of the enzyme such as influence of carbon/nitrogen sources, influence of agitator, influence of temperature, influence of inducer, pH, *etc*. [7]

Twenty-four fungal isolates that belonged to six genera (*Alternaria, Aspergillus, Cladosporium, Pencillium, Rhizopus* and *Trichoderma*) were tested for producing laccase enzyme, out of which *Trichoderma harzianum* showed maximum production at 35 ºC, pH 5 after a duration of 6 days. These all isolates were basically isolated from bio deteriorated ancient papers and parchment. There is an exception among fungal species that do not produce laccase, which includes zygomycetes and chytridiomycetes. After the screening of potent fungi producing enzymes, their standard inoculum was cultivated under fermentation conditions for proper growth and enzyme production. The experiments were done using different temperatures (20 ºC, 25 ºC, 30 ºC, 35 ºC, and 40 ºC) and pH ranging from 4 to 7 to enhance the production of enzymes. Screening of potent fungus producing laccase was done using 100ml of productive liquid medium containing 3g Peptone, 10g Glucose, 0.6g KH_2PO_4, and 0.001g $ZnSO_4$, 0.4g K_2HPO_4, 0.0005g $FeSO_4$, 0.05g $MnSO_4$ and 0.5g $MgSO_4$ per liter, pH 5.5. This was then kept under incubation at 30 ºC for 12 days on a rotary shaker (150 rpm).[12]

Laccase uses oxygen and produces water as a by-product; thereby it is not harmful to the environment. They exhibit a broad substrate range which varies from one laccase to another. This enzyme has been produced by many species of soft, white-rot fungi, geophilous saprophytic fungi, and by many edible mushrooms including the oyster mushroom *Pleurotus ostreatus*, the rice mushroom *Lentinula edodes* and champignon *Agaricus bisporus*. It has been reported that *Phanerochaete chrysosporium* NCIM1197 also secrete extra-cellular laccase. Among various inducers, copper sulfate was found to have the highest tendency to enhance the production of this enzyme. Moreover, the production rate increased by 3.5 folds in the presence of this inducer. The industrial-level production for this enzyme was done from white-rot fungi *Trametes pubescens* MB89. Techniques are being developed to express the production of laccase in crop plants. Some of the reported laccases have got the ability to perform the activity under critical conditions like the presence of a high concentration of chloride and cupric ions. Being under neutral pH laccase enzyme is said to be active in cuticle sclerotization in the case of insects.

They have been characterized in different insects *e.g. Bombyx, Calliphora*, *Diploptera,* and *Drosophila*. Maximal laccase activity was seen when white-rot fungus *Trametes pubescens* MB89 was used as the source of the production, using an optimized medium containing glucose (40g/l), peptone from meat (10g/l), $MgSO_4.7H_2O$ and 2.0mM Cu for stimulating enzyme formation. The commonly used carbon sources for producing

laccase enzyme include glucose, maltose, sucrose, fructose, glycerol and lactose, and the commonly used nitrogen sources includes yeast extract, peptone, urea, ammonium sulfate, ammonium nitrate. The excessive concentration of glucose and sucrose are inhibitory to the enzyme production in certain fungal strains. Studies show variation in laccase activity depending on the carbon to nitrogen ratio taken during the production procedure [13].

The influence of substrates during the fermentation process to produce enzyme plays a vital role. In *Streptomyces cyaneus*, a laccase type phenol oxidase was produced under solid-state fermentation conditions. Submerged fermentation is used frequently to produce laccase using fungi. Apart from pH, temperature and incubation period, the influence of carbon and nitrogen sources, metal ions, inducers also play a vital role in the production of this enzyme. The production time for laccase was standardized using composite minerals such as glucose and guaiacol, moreover, the productivity was found high in the medium that had rice and maize bran than glucose as a carbon source. It was found that solid-state fermentation served as an economical method of production of laccase by bacteria where cereal grains, wheat bran, sawdust, wood shavings and other plant and animal materials were used as substrates under this type of fermentation process, moreover the moisture content of 15% was also essential throughout. Both semi-solid and submerged fermentation acts as favorable method for different bacterial and fungal species for *e.g.* laccase enzyme productivity was high for *T. versicolor* under submerged fermentation whereas semi-solid conditions were more favorable for *T. villosa* [14].

The treatment of industrial effluents before discharge to the environment, was also done by laccase enzymes that are produced from almost every major source majorly including plants, fungus, insects, and bacteria. They are becoming highly essential in various aspects of controlling environmental pollution [15]. Apart from copper sulfate (1mM), inducers such as syringaldazine (0.11µM) were used in optimized conditions to enhance the production of laccase under solid-state conditions. Ground orange peelings were taken as substrate; these peelings serve as the source of both soluble and insoluble carbohydrates and the organism taken as a source was *Trichoderma hirusta*. The produced laccase enzyme showed the impact of both the amount of copper concentration and the amount of substrate on its activity. A fixed bed bioreactor and tray bioreactor were used for the production considering different pH ranges and different incubation periods. But it was concluded that the tray bioreactor had better configuration for the enzyme production by *T. hirusta* in solid-state conditions. Orange peelings (2.5g or 5g) on the experiment and amount of culture medium were used as a support substrate for producing laccase by *T. hirsuta* under solid substrate fermentation.

The substrate composition was subjected to basal culture medium containing per liter: 4g glucose, 15g Yeast extract, 0.75g NH_4Cl, 2g KH_2PO_4, 0.5g $MgSO_4.7H_20$, 0.1g $CaCl_2.2H_20$, 0.5g KCl, and 20Mm acetate buffer (pH 4.5). It was also reported that an optimal pH ranges from 5.0-5.5 under solid-state conditions was considered favorable for enzyme production by *T. hirsute* [16].

A mushroom species *Coriolus versicolor* was used to determine the ligninolytic activities of laccase enzyme. Solid-state fermentation was implemented for the process and solid substrate taken were rice bran and peanut shell. For efficient production of enzyme, various factors were investigated, including initial pH, incubation period, temperature, carbon, and nitrogen sources. Optimum pH range was taken from 4.5 to 7. Incubation was done at 37 ºC for a period of 12, 18, 24 36 and 42 days. The fermentation was carried out at a different range of temperatures (25ºC to 37 ºC). The nutrient sources preferred included 1%, 2%, 3%, and 5% glucose and chicken pea powder in 5g substrate. Defined medium with 3.0g/l Peptone, 10.0g/l Glucose, 1.0g/l KH_2PO_4, 0.001g/l $ZnSO_4$, 0.4g/l K_2HPO_4, 0.0005g/l $FeSO_4$, 0.05g/l $MnSO_4$, 0.5g/l $MgSO_4$ and 20g/l Agar were used for screening this fungal strain. 0.02% Guaiacol was supplemented to the medium, and it was left for incubation at 25 ± 1 °C for 7 days.

The adjustment of optimal pH was done using range from 4.5 to 7. Glucose and Chickpea powder were taken as carbon and nitrogen sources respectively. The glucose source was taken in different amounts (1%, 2%, 3%, and 5%) along

with nitrogen source as 5g substrate and was subjected to variations in incubation temperature from 25 °C - 37 °C) for 36 days [17].

Agaricus bisporus D621 which is a heterokaryotic fertile strain of mycelium was used as a source to produce laccase in a small scale culture using basal medium containing various carbon sources (1% w/v) – Glucose, Mannitol, Fructose, Trehalose, Xylose, Cellobiose, Maltose, Sucrose, Ribose, Rhamnose; Malt extract (2% w/v), L-glutamic acid 0.07% w/v as the nitrogen source which was neutralized with KOH, Thiamine (0.2mg/l) and Biotin (0.02mg/l) which served as the essential vitamin requirements. Cycloheximide at a concentration of 1.0 and 0.1mM was found inhibitory for both and production of the enzyme. The enzyme activity was focused on the pH range (3.4 – 4.0) [18].

The potent isolates of yeast *Kluyveromyces dobzhanskii* DW1 and *Pichia manshurica* DW2 that had the ability of producing laccase were screened using the glucose yeast extract peptone-Copper Sulphate broth medium which contained (in g/l): Glucose (10.0), Yeast extract (5.0), Peptone (3.0) and Copper sulfate (0.1) dissolved in citrate phosphate buffer of pH 5.0. The carbon sources (1%w/v) added were glucose, sucrose, maltose, fructose, and starch along with peptone, yeast extract, ammonium sulfate, sodium nitrate and potassium nitrate serving as nitrogen sources (0.3% w/v). The enzyme production was optimized by visualizing the effect of different pH (3.0, 4.0, 5.0, 6.0) and temperature (25 °C, 30 °C, 35 °C, and 37 °C). Apart from this various inducer were also added at a concentration of (0.02% w/v) including copper sulfate, tannic acid, catechol, hydroquinone, acetaminophen, pyrogallol that affected the productivity of enzyme. Higher laccase activity was obtained at the optimum pH of 6.0and incubation temperature of 30 °C and 35 °C [19].

Purification: Laccases are purified from the plant by its sap or tissue extract. Fungal laccases are purified from the culture medium (fermentation broth). Purification can be done in a single step or in a multistep process. The complete down streaming process was carried out in two steps; the primary step included solid/liquid separation by filtration and centrifugation. Salting out precipitation method using ammonium sulfate salt would dewater the sample. In the second step, ion exchange and gel filtration chromatographic techniques were done. Laccase was purified from *Neurospora crassa* by celtie chromatography and 54 fold purification with a specific activity of 333U mg-l. Single monomeric Laccase can be purified from *T. versicolour* by ethanol precipitation, DEAE Sepharose, Phenyl – Sepharose and Sephadex G-100 chromatography with a specific activity of 91, 433U mg^{-1}, also laccase was purified from *T. versicolour* by ion-exchange chromatography followed by gel filtration with a specific activity of 101U mL-1 and 34.8 fold purification [18].

Purification of enzymes is a major step in order to understand the structural and functional properties of an enzyme and to know its applications. The general methods used for the purification of enzymes are filtration, centrifugation, defiltration, precipitation, and chromatography (ion-exchange chromatography and gel filtration chromatography). For successful purification of enzymes, appropriate techniques must be followed [20].

MinaAllos purified laccase enzyme from *Bacillus cereus* by ammonium sulfate precipitation method, dialysis, ion-exchange chromatography, gel filtration chromatography, and gel electrophoresis. Ammonium sulfate precipitation involved the addition of ammonium sulfate to the crude laccase filter collected from *B. cereus* and then centrifugation at 6000 rpm for 20 min at 4 °C. The pellets got were dissolved in potassium phosphate buffer. This was then introduced into the dialysis tube and dialyzed overnight against the same buffer. This was then further purified by ion-exchange chromatography technique using DEAE-Cellulose column. Laccase enzyme was eluted out with a flow rate of 30ml/h. Laccase obtained was further purified by gel filtration chromatography technique through Sephacryl S-200. Laccase eluted out at 3ml/fraction using the same buffer. Gel electrophoresis of laccase was performed in the presence of SDS (SDS-PAGE). 20 µl of the sample was loaded on the gel and the total run time of the gel was about 4hr with cooling at 4 °C [21].

Laccase was purified from *B. tequilensis* SN4 by using acetone precipitation followed by column chromatography and by SDS-PAGE. 60% acetone

(chilled) was added to the crude enzyme and after some time the sample was centrifuged. The pellet was dissolved in 1M Tris-HCl buffer (pH 8.0). The precipitated proteins were applied to Sephadex G-150 column and eluted with buffers. They were then concentrated by polyethylene glycol (PEG) and then it was applied onto the DEAE-cellulose column. The proteins were then eluted with a linear gradient of NaCl. The enzyme purity was determined by running it on SDS-PAGE gel electrophoresis. The enzyme was found to be purified to 28-46-fold with a yield of 13.34%. The band in the SDS-PAGE was found to be of 32kDa [22].

Benney Chefetz *et al*., purified laccase from *Chaetomium thermophilium*. They took the supernatant of a 48 h culture and were filtered through a 0.45µm pore size membrane filter. Tangential flow membrane filter by using a 10kDa filter cassette and a pressure of 0.12MPa was used to concentrate the culture liquid. The concentrated culture was dialyzed overnight and loaded onto a DEAE-Sepharose anion-exchange column. The fractions containing laccase were concentrated with Amicon cell. It was again loaded onto a concanavalin A-Sepharose column and the purity of laccase was determined by gel electrophoresis [23].

Laccase was purified from the fungal species Pleurotus sp. The supernatant of the culture filtrate was precipitated by ammonium sulfate precipitation method. The precipitate was dialyzed and lyophilized and loaded onto a DEAE-Cellulose anion-exchange column followed by gel filtration chromatography by using a Sephadex G-100 column. Bradford's method was used to determine the amount of protein in the sample. Here also the purity of the sample was determined by performing gel electrophoresis. Laccase was purified to 72.2-fold with a yield of 22.4% [24].

Laccase was also purified from *Ganoderma lucidum*-CDBT1 by centrifuging the culture at high speed and subjecting the supernatant to filtration. The enzyme was then precipitated using ammonium sulfate precipitation, then followed by overnight dialysis. The enzyme was finally subjected to SDS and native PAGE to determine the purity of the enzyme [25].

Laccase enzyme was purified from the yeasts, *Kluyveromyces dobzhanskii* and *Pichia manshurica* isolated from ligninolytic soil. Ammonium sulfate precipitation was used to purify the crude enzyme followed by centrifugation. The resultant solution was dialyzed. The desalted enzyme was further applied to a Sepharose gel G-100 fast flow column pre-equilibrated with pH 5.0 [26].

Laccase from the white-rot fungus, *Marasmius scorodonius* was purified by ammonium sulfate precipitation after filtration and centrifugation of the culture. It was then dialyzed and concentrated by ultrafiltration by using a YM-10 membrane. The concentrated proteins were further purified in HiTrap Q HP column and washed with acetate buffer. The proteins were then separated by SDS-PAGE on 10% gels [27].

D. A. Wood purified laccase extracellular laccase from *Agaricus bisporus.* The bulk culture filtrates were first ultra-filtrated and then precipitated using ammonium sulfate. The precipitate was then dialyzed for 24 h after dissolving in phosphate buffer. This was then applied to the top of a column of DE52 ion-exchange cellulose. The column was further developed by a linear gradient of 0 to 0.5M NaCl. The sample was then layered on top of Sephadex G-100 column. Fractions obtained from the column were further layered on top of Concanavalin A-Sepharose [28].

Ammonium sulfate precipitation followed by dialysis and column chromatography was done in order to purify laccase from *Aspergillus nidulans* [29].

TABLE 2: PURIFICATION STEPS OF LACCASE ENZYME FROM DIFFERENT SOURCES

S. no.	Source	Step	Enzyme activity	Protein conc. (mg/ml)	Specific Activity (U/mg)	Total activity (U)	Purification fold	Yield (in %)	Molecular Weight of Laccase from SDS PAGE(kDa)
1	*Bacillus cereus*	Crude enzyme	1400	0.2	70.00	140000	1	100	66
		Ammonium precipitation	3400	0.16	21.250	102000	3	72.8	

		Ion exchange chromatography	5000	0.05	100.00	75000	14.2	53.5	
		Gel filtration chromatography	4600	0.02	230.00	230.000	32.8	49.2	
2	*B. tequilensis* SN4	Acetone precipitation	3599.28	195	18.46		1.75	63.38	32
		Sephadex G-150	1507.52	15.79	95.47		9.07	26.55	
		DEAE-Cellulose	757.49	2.33	299.40		28.46	13.34	
3	*Chaetomium thermophilium*	Ultrafiltration	-	0.016		19	3	84	77
		DEAE	-	0.036		17	5	79	
		Concanavalin A	-	0.190		8.4	10	40	
4	*Pleurotus* sp.	Ammonium sulphate fraction	-	1300	107	18000	2.97	62	40
		DEAE cellulose chromatography	-	125	1300	14500	12.14	50	
		Sephadex G-100 chromatography	-	12	2600	6500	72.2	22.4	
5	*Ganoderma lucidum*-CDBT1	Ammonium sulphate precipitation	-	35.9	86.85	3115.74	94.4		43
		DEAE-anion Exchange chromatography	-	13.3	201.00	2662.04	218.0		
6	*Kluyveromyces dobzhanskii*	Ammonium Sulphate precipitation	-	0.120	0.694	0.083	3.521	87.5	-
		Dialysis	-	0.119	0.639	0.076	3.245	82.8	
		Sephadex G-100 chromatography	-	0.083	0.410	0.034	0.410		
7	*Pichia manshurica*	Ammonium Sulphate precipitation	-	0.159	0.500	0.080	3.743	88.8	-
		Dialysis	-	0.157	0.505	0.079	3.776	83.2	
		Sephadex G-100 chromatography	-	0.131	0.450	0.059	3.369		
8	*Marasmiusscorodonius*	Ammonium sulphate precipitation	-	127	6.3	802	4	17	67
		HiTrap Q	-	14	432.8	258	206	5	
9	*Agaricus bisporus*	Ultrafiltration	-	5.20	0.630	3.28	2.7	88	53.1
		Ammonium Sulphate precipitation	-	15.00	1.320	19.8	5.8	84	
		DEAE- cellulose chromatography	-	18.00	7.200	129.7	31.4	60	
		Sephadex G-100 gel filtration	-	6.56	13.250	80.3	57.8	42	
		Con A-Sepharose affinity chromatography	-	7.24	13.700	99.2	59.8	38	
10	*A. nidulans*	Ultrafiltration	-	12	11125		34	545	66
		Ammonium Sulphate Precipitation	-	150	4.56		1.35	62.9	
		DEAE - Cellulose	-	60	7.16		2.95	50.5	
		Biogel P-200 Laccase enzyme	-	30	9.19		5.8	42.45	

CONCLUSION: It was found that several extant ectomycorrhizal fungal taxa possessed laccase like genes which showed strong nucleotide sequence similarity to laccase genes in white-rot fungi. Multiple laccase-like genes were identified in Piloderm [21].

Laccase is a kind of polyphenol oxidase that contains copper atoms and they can oxidize an array of organic and inorganic substrates by the reduction of oxygen to water. The white-rot fungus Trametes sp. AH28-2 can synthesize extracellular laccase by induction in the cellobiose-based liquid culture medium. The Trametes sp. had increased production of laccase enzyme when they were treated with inducers - small-molecule aromatic compounds, o-toluidine, guaiacol and 3, 5-dihydroxytoluene. The production of laccase enzyme decreased when Trametes sp. were co-cultured with other bacteria. The optimum pH for the growth of Trametes sp. and its production of Laccase enzyme was found to be at a pH of 3.5 to 7.5 [22].

Laccase enzyme can also degrade the activity of cercosporin and reduce its toxicity toward living cells. Cercosporin generally causes membrane damage and mortality of living cells [23].

The novel isoform of Laccase produced by *Marasmius quercoph* a white-rot fungus, isolated from cork oak litter and this enzyme could potentially be applied in various detoxification processes such as pesticide removal or industrial phenolic wastewater treatment [24].

Pycnoporus sp. SYBC-L3 could produce a novel thermally stable laccase (lac-L) which was different from other laccases in terms of molecular mass, temperature optima and stability, pH optima and stability, and substrate specificity. Lac-L had great potential in dye enzymatic decolorization. Hence Lac-L can be used for industrial and environmental applications [25].

Laccase secretions and activities are used by necrotrophic fungi, *Botrytis cinerea* to neutralize the toxicity of grape *Stilbenic phytoalexins*, thus the grape pathogenesis-related proteins become insoluble [26].

Laccase is an important enzyme that has a major role in the Arabidopsis laccase family which confirmed 20 true mutants for the 12 laccase genes out of a total of 17 laccase genes and three of these mutants showed typical phenotypic characters [27].

Laccase helped in the co-evolutionary adaptation in the fungal symbiont and it was found to help the leaf-cutting ants to overcome plant defensive phenolic compounds [28].

The study of laccase genes at the cDNA levels is an important prospect for future studies because the cDNA DNA-level approaches on laccase enzyme presented a good consistency of diversities but different compositions of laccase-containing basidiomycete communities [29].

Laccase enzyme was found to be responsible for epicatechin mediated anthocyanin degradation using Litchi fruit as the specimen which gives the possibility of identification of similar enzymes that are responsible for anthocyanin degradation in other fruits after harvest [30].

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