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PRODUCTION, PURIFICATION AND BIOTECHNOLOGICAL APROACH OF LACCASE ENZYME: A BRIEF OVERVIEW

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ABSTRACT

Laccases have drawn the attention of researchers everywhere due to their exceptional features and applicability in food industry. Laccase is a copper-based enzyme and it belongs to oxidoreductases. There are many sources for laccase isolation but for commercial applications, microbial source (bacterial, fungal and algal) are often used. Some bacterial species, like *Pseudomonas sp*. and *Bacillus sp.* are known as the best producer of above said enzyme. For industrial scale production of Laccase, solid state (SSF) and submerged fermentation (SMF) is being used widely. Different purification techniques such as ammonium sulfate precipitation, dialysis, Ion exchange chromatography as well as size exclusion chromatography are employed to obtain high yields of enzyme. Laccase can react with a huge spectrum of carbolic acid and non-carbolic acid compounds and that is why the laccase-mediator system was developed as a biological substitute for chlorine bleaching. Several types of contaminants could be detoxified by using laccase enzyme by acting on a variety of substrates, making them useful in industries such as paper, pulp, and textiles. Near term recombinant laccase creation would benefit from a reduction in the series of steps required for enzyme pre-purification, as well as high yield, increased activity, and stability. This review article discusses the laccase enzyme's sources, production, conditions, purification techniques as well as its biotechnological applications.

Key words: Laccase, production, bacterial sources, fermentation, *Bacillus* sp., laccase mediator, industrial applications

INTRODUCTION

In the industrial world, enzymes play an integral role because they increase the accessibility of catalyst for down-stream reactions, which speeds up reactions and improves yield. Laccase is among the most widely used enzymes in the industrial sector due to its versatility (Chauhan *et al*., 2017). Laccases, also known as green enzymes, belong to multicopper oxidases which have copper-binding domains that are rich in histidine (Ihssen *et al*., 2015). It is a glycoprotein containing a carbohydrate content of 15 to 30% with a molar mass ranging from 60 to 90 kDa (Ashraf *et al*., 2020).

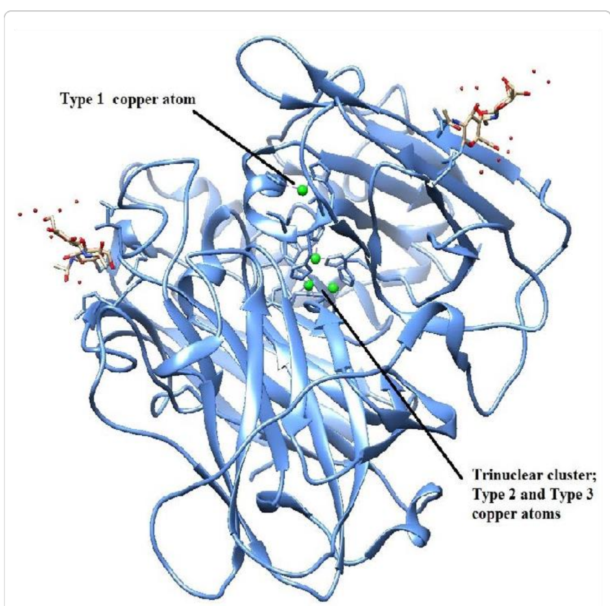

Fig. 1.Crystal Structure of laccase (Guest *et al*., 2016).

With the assistance of laccase, a variety of oxybenzene compounds undergo one electron oxidation (Jones *et al*., 2015) utilize molecular O (atomic number 8) as a cosubstrate (Ernst *et al*., 2018) and yields water as a derivate (Chandra and Chowdhary *et al*., 2015). Laccase mediators can react with oxybenzene substances (Hakulinen *et al*., 2015) and degrades lignin by 80–90% with the assistance of these mediators. Laccases, unlike many oxidoreductases and peroxidase, neither need co-factors like NADPH and nor produce toxic peroxide intermediate (Mehra *et al*., 2018).

BACTERIAL SOURCES ARE BEST PRODUCERS OF LACCASE

Bacterial laccase embrace enzymes are more efficient than fungal laccase due to the relatively low redox potential (Arregui *et al*., 2019) and their survival in unfavorable conditions like those of high thermal stability, wide pH variations, and high sodium concentrations (Sharma *et al*., 2017). Furthermore, bacteria grow faster in liquid media than fungi, which enhances the yield of laccase (Lanfang *et al*., 2021).

SOURCES OF LACCASE ENZYME

Laccases are abundant in nature and yielded by many plants and different microbes.

Table 1. Plants and Microbes as protentional laccase source.

Source	**Organism**	**References**
Plants	*Pyrus Mallus, Cruciferous plant, Pyrus Bradford, Solanum tuberosum L,* and other vegetables	(Ashraf *et al*., 2020)
Fungi	*Deuteromycetes, Basidiomycetes, Ascomycetes* and 60 other fungi	(Patel *et al*., 2019)
Bacteria	*Bacillus subtilis, Azospirillum lipoferum, Saccharomyces cyaneus, Pseudomonas pyocyanea*	(Ashraf *et al*., 2020)

METHODS OF PRODUCTION

Enzyme is produced by using bacteria as inoculum and various substrates (Dassi *et al*., 2016). Inoculum was prepared in the laboratory. Then optimization is achieved by using substrate and employing Mineral Salt solution, as well as using multiple variables like source of nitrogen and carbon, pH, temperature and varied incubation times (Mishra *et al*., 2016).

After incubation, the appearance of a zone along the optimal wells served as evidence that laccase had been produced. The homogenate is used to determine high laccase activity using a UV spectrometer (720nm) (Akpinar *et al*., 2017).

Laccase is synthesized by two modes of fermentation.

Submerged Fermentation (SMF)

In submerged fermentation, microorganisms produce enzymes in a liquid nutrient medium (Ferootanfar and Faramarzi, 2015). Submerged fermentation produced more growth and laccase synthesis due to the pre-determined bioreactor configurations and ease of parameters control (Xu *et al*., 2017).

Solid State Fermentation (SSF)

Solid substrates like sugarcane bagasse, rice and wheat bran are utilized in solid state fermentation to cultivate microbes and manufacture enzymes (Sharma *et al*., 2017)

Table 2. Some bacteria that produce laccase along with mode of production and parameters used.

Microbial Source	Source of carbon	Production mode	Substrate used	Optimal T (°C)	Optimal pH	References
Bacillus subtilis MTC 2414	Agro-waste	SSF	Guaiacol	30-40 °C	7.0	(Chauhan *et al*., 2017)
Lysinibacillus sphaericus strain LH3.4	M16 medium	Centrifuge at 150rpm	ABTS	32°C	NR	(Kaur *et al*., 2016)
Bacillus sp. *WT*	SWN medium	SSF	ABTS	37°C	5.0	(Janusz *et al*., 2020)
Aquisaibacillus elongatus	LB medium	SSF	DMP	40°C	8.0	(Rezaei *et al*., 2017)

VARIABLES THAT AFFECT THE SYNTHESIS OF LACCASE

The synthesis of Laccase was examined using a variety of optimization parameters (Guan *et al*., 2018). Laccase production can be facilitated by using fructose as a carbon source, low carbon-to-nitrogen ratio and its activity is best at 30–40 °C. PH of 6-7 is ideal for laccase production (Jhuang *et al*., 2020). Laccase production was inhibited by higher glucose concentrations in various strains.

PURIFICATION TECHNIQUES

Laccases are purified from plants using sap or tissue extract. The culture medium is used to purify fungal laccases (Umar.A *et al*., 2022).

Filtration, centrifugation, precipitation, and chromatography are the methods used to purify bacterial laccase enzyme (Nivedharshini, 2020). Ammonium sulphate was combined with the crude laccase filtrate, and the mixture was then incubated at 4°C for the entire overnight period. The same buffer was employed to dialyze the precipitate at 4°C after being centrifugally filtered at 8000 rpm for 20 minutes. The DEAE-Cellulose column was then used to purify it further using an ion-exchange chromatography technique. Having a rate of flow around 30 mL/h, the laccase enzyme was eluted out. Laccase was purified further using a Sephadex-based gel filtration chromatography technique (Bagewadi *et al*., 2017).

Using the same buffer, laccase was eluted at 3 mL/fraction. In the presence of SDS, gel electrophoresis was carried out. The gel was filled with 2.0 L of sample, and the whole run time was around 4 h included cooling at 4 °C.

Table 3. Different Purification methods and laccase enzyme activity.

Purification Steps	Enzyme activity	Ratio of a activity units to amount of protein (U/mg)	Protein (mg/ml)	Purified (Fold)	Yield Recovery (percent)	Reference
Separation of Raw material	1400	70	0.2	1	100	(Forootanfar *et al*., 2019)
Ammonium Sulphate Precipitation	3400	21.25	0.16	3	72.8	(Nivedharshini, 2020)
Ion-exchanged chromatography	5400	100	0.05	142	53.5	(Ezike *et al*., 2020)
Gel Filtration	4600	230	0.02	28	49.2	(Kumar *et al*., 2020)

BIOTECHNOLOGICAL APPLICATIONS OF LACCASE

Laccase has diverse industrial uses such as the removal of industrial harmful substances, acting as a biosensor to detect morphine, codeine, and catecholamine, and as kind of a bio-remediation weapon to alleviate weeds and specified chemicals from soil.

Laccase-mediated enzymatic treatment is thought to be an effective process for the decolorization (Neifar *et al*., 2016) of dye-contaminated industrial effluents (Zhang *et al*., 2020), improving and texturizing food quality at a low cost, bio-bleaching of synthetic dyes, and seems to have nanobiotechnological applications (Wehaidy *et al*., 2019).

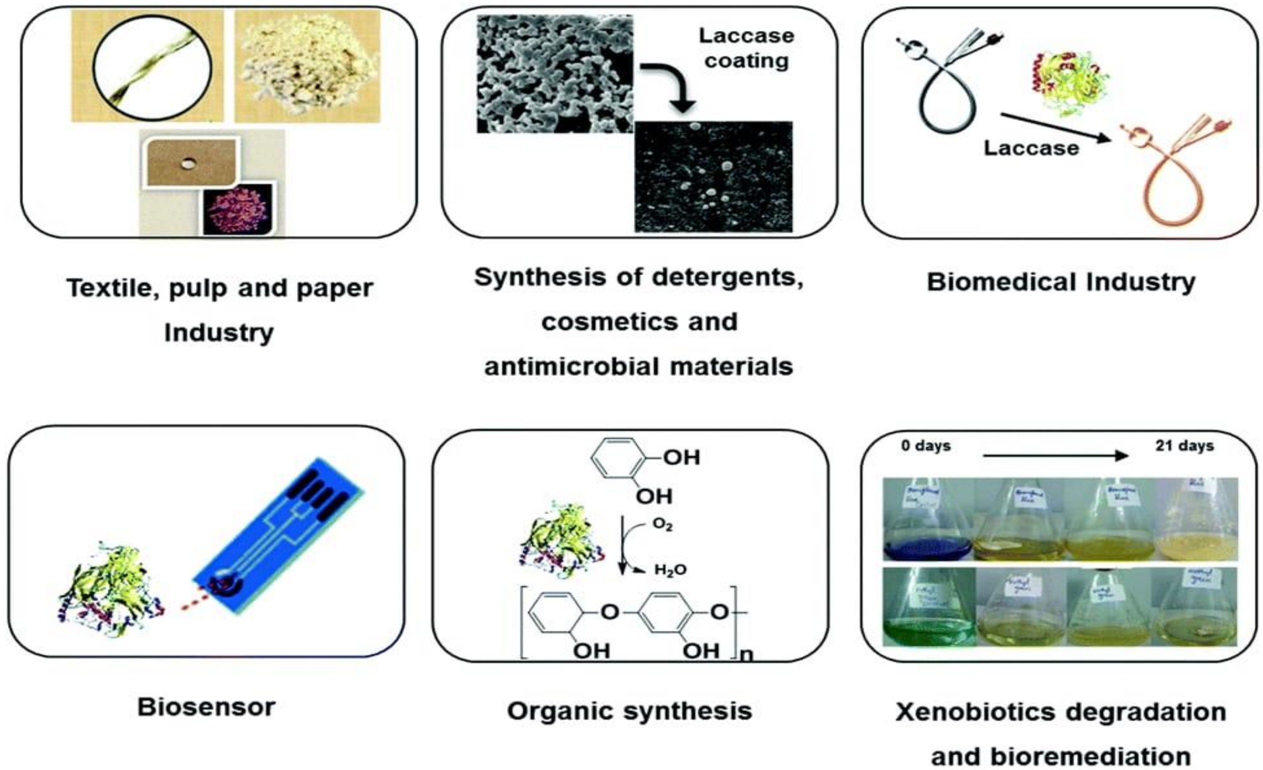

Fig. 2. Novel Biotechnological applications of laccase (Idalina *et al*., 2015).

Food Industry

Laccases are used in a variety of food processing applications, including juice extraction, beverage preservation, and baking (Singh *et al*., 2020). Laccases are used to preserve the color, taste, and quality of juice. Laccase improves crumb structure, volume, stability, strength, baked product softness, and stickiness (Bilal *et al*., 2019). According to reports, treating wine with laccase is a viable option for improving the characteristics of wines over extended periods of time, lowering manufacturing expenses and preventing degeneration.

Micro-fuel

Laccase is used as a cathode in micro-fuel cells (Mano and de Poulpiquet, 2018) to reduce deoxygenate.

Bioremediation

Laccases could be used for the treatment of contaminated soils because immobilised laccases can oxidize toxic organic pollutants like chlorophenols, polyaromatic, lignin-related structures (De Gonzalo *et al*., 2016), organophosphate compounds, phenols, and basic dyes. Laccases that have been immobilised are effective biodegradation agents.

Laccase is immobilised in NO_3 or boric acid cross-linked plasticizers. Because of their ligninolytic activity, laccases are considered eco-friendly degradative agents (Lee *et al*., 2022). The aromatic polycyclic compounds produced from non-renewable wastes and crude oil reserves as well as chlorinated phenolic contaminants deteriorate by this enzyme, which increases laccase activity in plant tissues as their concentration in the soil rises.

Bioelectrocatalysis

The innovation of biofuel cell cathodes and nanosensors is related to laccase's employment in bioelectrocatalytical mechanisms (Khan *et al*., 2019), enabling laccase a more ecologically friendly fuel cell material than chemical oxidation-based fuel cells.

Medicinal and health care applications

Laccases are biomolecules that act specifically; as a result, manufacturers use this enzyme to synthesize complex medicinal compounds (Jasim, 2017) like anesthetics, anti-inflammatory drugs, antibiotics, depressants, and so on. Iodide can be converted by laccase into the ubiquitous bactericidal iodine. To enlighten the skin, many skin-care products that are laccase-based hair colorants are used. The advantage of using these hair colorants is that they don't cause irritation and are safer than conventional hair colors (Antonio *et al*., 2019).

Protein recombinant laccase can be used to make deodorants, toothpaste, mouthwash, detergents, and soaps that are less allergenic.

Herbal treatment

Laccase, derived from the oyster mushroom (*Pleurotus ostreatus*), is being used as an herbal medicine (Jasim, 2017) to slow the replication rate of the HCV (hepatitis C virus).

Paper and pulp industry

Bio-bleaching with enzymes is a sustainable method of decolorization in the paper making mill (Singh *et al*., 2019). A fungus that is known as *Phanerodontia chrysosporium* produces laccase that reduce the level of lignin in order to increase the production of paper. Inorganic mediators may be substituted for organic ones to lessen the harshness of the procedure while still improving the potency of bio-bleaching with laccase (Mao *et al*. 2021). The utilization of a mixture of hemicellulose and ligninolytic enzymes could improve pulp quality while lowering chlorine levels (Sharma *et al*. 2020).

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