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Optimization and characterization of a novel laccase from *Geotrichum candidum* Link for sustainable dye decolorization

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Abstract
The increasing environmental burden of synthetic dyes and lignin-rich waste has intensified the demand for sustainable biocatalysts, particularly microbial laccases, for bioremediation and industrial applications. While white-rot basidiomycetes dominate laccase research, their slow growth, pathogenic potential, and low yields under industrial conditions necessitate exploration of alternative fungal sources. This study addresses this gap by isolating a novel *Geotrichum candidum* Link strain (GenBank: KJ814246) from tropical laterite soils—a promising yet underexplored reservoir of ligninolytic microbes. The fungus was selected for its GRAS (Generally Recognized as Safe) status and robust laccase production (253.45 U/L), optimized through Response Surface Methodology, representing a 1.57-fold enhancement over basal conditions. The 62 kDa monomeric laccase exhibited unique dual pH optima (pH 5 for ABTS, pH 6 for syringaldazine) and retained >80% activity at 40°C, demonstrating operational versatility. With a K_m of 0.637 mM for ABTS, the enzyme outperformed several fungal laccases in dye decolorization, achieving 92% methyl red and 81% Congo red degradation within 3 hours without mediators—a critical advantage for industrial wastewater treatment. Statistical optimization identified malt extract (4.39 g/L) and pH 6.21 as key production parameters, while inhibition studies confirmed classical copper-dependent catalysis (54% inhibition by NaN_3). This work establishes *G. candidum* Link as a sustainable alternative to traditional laccase producers, addressing two key industry demands: (1) non-pathogenic, high-yield enzyme sources compatible with mild processing conditions, and (2) efficient, mediator-free dye degradation systems aligned with EU's 2024 wastewater mandates. The findings highlight the untapped potential of soil-derived deuteromycetes in circular bioeconomy strategies.

Keywords: Azo dyes, bioremediation, fungal enzymes, response surface methodology, textile effluent

Introduction

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are versatile multicopper oxidases that catalyze the oxidation of diverse phenolic and non-phenolic substrates, with water as the only by-product (Claus, 2004) [1]. Initially discovered in the Japanese lacquer tree *Rhus vernicifera*, these enzymes are now known to be widely distributed across plants, fungi, bacteria, and insects (Kunamneni *et al*., 2007) [2]. Among microbial sources, fungi—particularly white-rot basidiomycetes—have emerged as the most efficient producers due to their high redox potential and broad substrate specificity (Baldrian, 2006) [3]. Fungal laccases play pivotal roles in lignin degradation, morphogenesis, pigment biosynthesis, and detoxification of phenolic compounds, making them valuable for industrial and environmental applications (Mayer and Staples, 2002) [4].

Despite extensive research on laccases from basidiomycetes, significant gaps remain in understanding their production by deuteromycetous fungi, particularly under optimized conditions. Most studies have focused on a limited number of fungal species, leaving the potential of soil-derived isolates largely unexplored (Strong and Claus, 2011) [5]. Additionally, while laccase-mediated dye decolorization has been demonstrated, the efficiency varies substantially across fungal species and dye types, necessitating further exploration of novel strains with enhanced catalytic properties (Bilal *et al*., 2017) [6]. Another critical gap is the lack of comprehensive studies combining statistical optimization of production parameters with detailed enzyme characterization and application testing, which is essential for industrial scalability (Senthivelan *et al*., 201).

The growing demand for laccases in bioremediation, textile dye decolorization, pulp bleaching, and biosensor development has intensified research into novel microbial sources and optimized production strategies (Couto & Toca-Herrera, 2006) [8]. While basidiomycetes have been extensively studied, reports on laccase production by deuteromycetous fungi such as *Geotrichum candidum* Link remain scarce (Zhao *et al*., 2012) [9]. This study aimed to isolate a novel laccase-producing fungal strain from soil samples and characterize its potential for industrial applications. The objectives included molecular identification of the isolate using 18S rRNA gene sequencing, optimization of laccase production through statistical approaches like Plackett-Burman design and Response Surface Methodology, and evaluation of its dye decolorization efficiency for environmental applications.

Laccase production in fungi is influenced by various factors including pH, temperature, carbon and nitrogen sources, and the presence of inducers such as copper ions and aromatic compounds (Niladevi *et al*., 2009) [10]. Statistical optimization tools have proven effective in enhancing enzyme yields by identifying critical parameters and their interactions (Arockiasamy *et al*., 2008) [11]. The purified enzyme was characterized for its biochemical properties, and its potential in degrading synthetic dyes from textile effluents was assessed. Given the environmental persistence and toxicity of industrial dyes, microbial laccases offer a sustainable solution for wastewater treatment (Gupta & Suhas, 2009) [12]. This work highlights the untapped potential of soil-derived fungi, particularly *Geotrichum* species, as promising sources of industrially relevant enzymes for bioremediation and other biotechnological applications.

Materials and Methods

Isolation of the Fungal Strain

Fungi were isolated from moist soil collected from Thiruvananthapuram district, Kerala, using the serial dilution method (10^{-3} to 10^{-6} dilutions) and pour plate technique on potato dextrose agar (PDA) medium (200 g/L potato, 20 g/L glucose, 20 g/L agar) at 25°C (Gupta VK & Suhas, 1981). The plates were incubated at 30–35°C for 5–9 days, and fungal colonies were subcultured to obtain pure monocultures (Pepper *et al*., 1995) [14]. Pure cultures were maintained on PDA slants and stored at 4°C for further study (Aneja, 2020) [15].

Screening for Laccase Production

The fungal isolates were screened for phenol oxidase activity using tannic acid (0.5 g/10 mL, pH 4.7) incorporated into MMN agar (without malt extract). Degradation was indicated by a color change from pink-grey to brown (Bavendamm reaction) after incubation at 45°C (Giltrap, 1982) [16]. For laccase detection, isolates were cultured on PDA containing 2% guaiacol, with positive reactions identified by reddish-brown zones due to guaiacol oxidation (Reddy *et al*., 2008) [17].

Taxomorphological Characterization of Fungal Isolate

The fungal isolate was examined microscopically after staining with lactophenol aniline blue. Identification was based on conidiophore structure, conidial morphology (septation and pigmentation), and compared with standard taxonomic keys (Subramanian, 1971) [18]. The morphological identification was further confirmed by an expert mycologist, Dr. Hosa Gowda, Retired Scientist, JNTBGRI.

Molecular Identification of Fungi

The fungal isolate was identified by amplifying the 18S rDNA region using primers U18SF (5'-ACCTGGTTGATCCTGCCAG-3') and U18SR (5'-TGATCCTTCYGCAGGTTCAC-3'). Genomic DNA was extracted using a Sigma-Aldrich fungal DNA extraction kit, followed by purification and sequencing. PCR was performed in a 25 µL reaction mix (Table 2) under optimized conditions (Table 3). The amplicon was resolved on 0.8% agarose gel, purified, and sequenced on an ABI3730xl Genetic Analyzer. The sequence was analyzed using BLAST (NCBI) and phylogenetic reconstruction was performed via the Neighbor-Joining method (MEGA4) with 500 bootstrap replicates (White *et al*., 1990; Tamura *et al*., 2004; Tamura *et al*., 2007) [19, 20, 21].

Table 1: GenBank accession numbers of closely related *Geotrichum candidum* Link strains

S. No.	Name of strain	Accession No.
1	*G. candidum* Link strain GC	KJ814246
2	*G. candidum* Link clone AS F-1	KJ543497
3	*Galactomyces geotrichum* strain LMA-70	JQ668740
4	*Galactomyces geotrichum* strain PAMF3	JN252305
5	*G. candidum* Link	AB000652
6	*Galactomyces geotrichum* strain LMA-1025	JF262196
7	*Dipodascus macrospores*	AB000640
8	*Geotrichum carabidarum* strain Y-27727	AY520162
9	*Geotrichum histeridarum* strain Y-27730	AY520216
10	*Candida pallodes* strain NRRL Y-27914	DQ173676
11	*Candida tritomae* strain BG98	AY242146

Partial Characterization of Laccase from *Geotrichum candidum* Link

Laccase production was induced in *G. candidum* Link using a liquid medium (starch 20 g/L, yeast extract 2.5 g/L, mineral salts, pH 5.5) incubated for 9 days (Sivakumar *et al*., 2010) [22]. Crude enzyme activity was assayed via ABTS oxidation (ε420 = 36,000 $M^{-1}cm^{-1}$) at 420 nm in sodium acetate buffer (pH 5.0, 30°C) (Mtui & Masalu, 2008) [23]. Lignin peroxidase (LiP) activity was measured by veratryl alcohol oxidation (ε310 = 9,300 $M^{-1}cm^{-1}$) at 310 nm (Tien & Kirk, 1988) [24]. One unit (U) of enzyme activity was defined as 1 µmol substrate converted per minute. MnP activity was determined by monitoring guaiacol oxidation (ε465 = 12,100 $M^{-1}cm^{-1}$) at 465 nm in sodium succinate buffer (pH 4.5, 30°C). The reaction mixture contained 1 mM $MnSO_4$, 4 mM guaiacol, and 1 mM H_2O_2. One unit (U) of MnP activity was defined as the amount of enzyme converting 1 µmol guaiacol per minute (Wariishi *et al*., 1992) [25].

Optimization of Laccase Production by *Geotrichum candidum* Link

The optimal cultivation time for laccase production was determined by monitoring enzyme activity every 2 days. pH optimization (4.0–9.0) and substrate concentration effects (1.25–40.0 mM ABTS) were studied, with K_m and V_{max} calculated using EZ-Fit 5.03. Chemical inhibitors (NaN_3, NaCl, EDTA, L-cysteine; 0.125–0.750 mM) were tested for their inhibitory effects. Biomass determination involved drying mycelia at 105°C for 2 h and recording dry weight (Haq & Daud, 1995) [26].

Optimization of Laccase Production Using Response Surface Methodology

Laccase production by *Geotrichum candidum* Link was optimized using response surface methodology (RSM) with a basal medium (glucose 15 g/L, $(NH_4)_2SO_4$ 2.2 g/L, KH_2PO_4 1.6 g/L, $MgSO_4$ 0.3 g/L, $CaCl_2$ 0.07 g/L, KCl 0.07 g/L, malt extract 5 g/L, pH 7) supplemented with 0.1 mg/L $CuSO_4$ as an inducer (Plackett & Burman, 1946) [27]. A full factorial design (36 runs) was employed using MINITAB 16, with variables tested at low, center, and high levels. Laccase activity was assayed via syringaldazine oxidation (A530 nm, pH 6.5), with one unit (U) defined as 2 µmol substrate oxidized per minute (Ride, 1980) [28].

Table 2: Variables with low, centre and high set points

Medium components	Level (g/L)		
	Low	Centre	High
Glucose	15	20	25
$(NH_4)_2SO_4$	1.6	1.9	2.2
KH_2PO_4	1.6	1.9	2.2
$MgSO_4$	0.3	0.5	0.7
$CaCl_2$	0.07	0.05	0.13
KCl	0.07	0.05	0.03
Malt extract	3	4	7
pH	5	6	7

Table 3: Central Composite Design with observed and predicted values of laccase production by *G. candidum*

C1 (Malt extract)	C2 (pH)	Response observed U/L	Response predicted U/L	Residual
5	7	180.51	201.34	-20.83
4	6	253.71	244.20	9.51
5	5	171.31	180.83	-9.52
4	6	243.81	244.20	-0.39
2.59	6	106.31	118.61	-12.3
5.41	6	232.4	212.51	19.89
4	6	242.3	244.20	-1.9
4	6	242.9	244.20	-1.3
3	5	93.45	80.20	13.24
4	4.59	107.3	111.50	-4.2
4	6	238.3	244.20	-5.9
4	7.41	200.7	188.91	11.79
3	7	171.1	169.17	1.93

Purification and Characterization of Fungal Laccase

Laccase from *G. candidum* Link was purified through ammonium sulfate precipitation (80% saturation), dialysis (3-kDa cutoff), and sequential chromatography (HiTrap Q FF anion exchange, Superdex 75 gel filtration). Active fractions were pooled, concentrated, and analyzed by SDS-PAGE (12% gel) for homogeneity (Zouari-Mechichi *et al*., 2006) [29]. Protein content was determined via the Lowry method, and laccase activity was assayed using syringaldazine oxidation (A530 nm) (Lowry *et al*., 1951) [30]. The enzyme demonstrated textile dye decolorization (methyl red, Congo red), with % decolorization calculated as (Saranyu & Rakrudee, 2007) [31]:

$$\% \, Decolorization = \frac{A0 - At}{A0} \times 100;$$

where $A0$ = absorbance (control), At = absorbance (enzyme-treated)

Characterization of Purified Laccase from *Geotrichum candidum* Link

The purified laccase was evaluated for thermal stability by pre-incubation at 20–60°C (15–60 min), with residual activity measured. pH stability was tested in buffers (pH 3–10), while metal ion effects (Mg^{2+}, Mn^{2+}, Fe^{2+}, Hg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}; 1–10 mM) were assessed after 15 min pre-incubation (Yang *et al*., 2017) [32].

Results

Isolation and Screening of Fungi for Laccase Production

Fungal strains were successfully isolated from soil samples collected in Thiruvananthapuram, Kerala, using the pour plate method with serial dilutions (10^{-3} to 10^{-6}) on PDA medium. Screening for phenol oxidase activity revealed a color change (pink-grey to brown) in tannic acid-amended agar, confirming phenolic degradation via the Bavendamm reaction. For laccase detection, fungal isolates cultured on guaiacol-supplemented PDA produced reddish-brown oxidation zones, indicating enzymatic polymerization of guaiacol. The absence of hydrogen peroxide requirement confirmed the presence of true laccase. (Fig 1a, b)

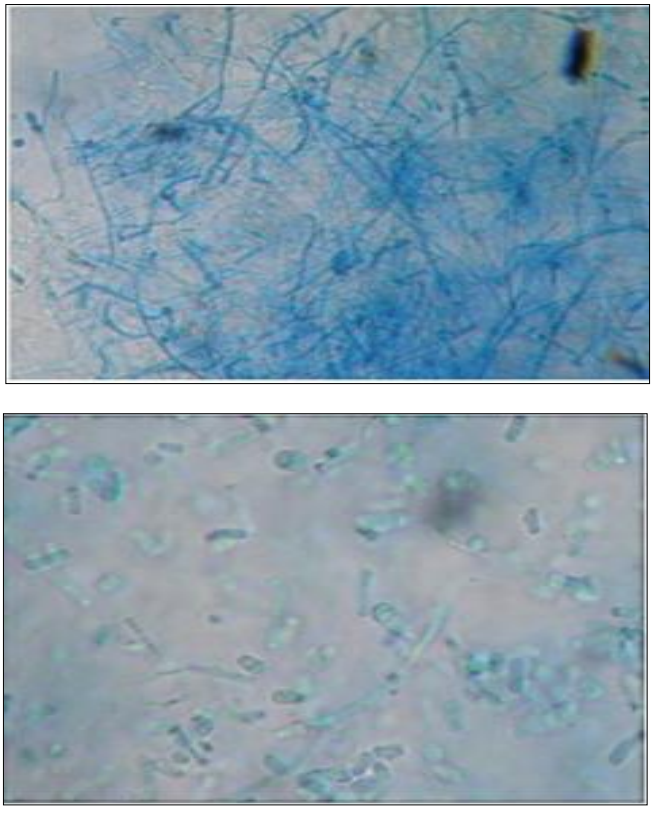

Fig 1: a- Hyphae of *Geotrichum candidum* Link b: Spore of *Geotrichum candidum* Link

Taxomorphological Characterization of the Fungal Isolate

The isolated fungus exhibited characteristic features of *Geotrichum candidum* Link, including white, powdery, dome-shaped colonies with a yellowish center and branched, septate hyphae. The fungus produced arthroconidia (4–11 × 1–2 µm), a defining trait of *Geotrichum*. Initially classified under Deuteromycetes, modern taxonomy places *G. candidum* Link within the Hemiascomycetes (Ascomycota), with the current classification as follows:

Kingdom: Fungi
Phylum: Ascomycota
Class: Hemiascomycetes
Order: Saccharomycetales
Family: Candidaceae
Genus: *Geotrichum*
Species: *candidum* Link

The isolate's morphological and reproductive traits align with previous descriptions of *G. candidum* Link, confirming its identity.

Etiology of *Geotrichum candidum* Link

Geotrichum candidum Link is a versatile fungus with significant ecological, clinical, and industrial implications. In the environment, it is commonly found in soil, water, decaying vegetation, and dairy products, where it plays a role in organic matter decomposition and food fermentation. Notably, it is utilized in the ripening of certain cheeses, contributing to flavor and texture development. However, despite its beneficial uses, *G. candidum* Link can also act as an opportunistic pathogen, particularly in immunocompromised individuals. Clinical infections, termed geotrichosis, may manifest as pulmonary, oral, cutaneous, or systemic diseases, often resembling other fungal infections such as candidiasis. The fungus can enter the body through inhalation of spores, ingestion of contaminated food, or direct contact with broken skin. Risk factors for infection include prolonged antibiotic use, immunosuppression (e.g., HIV/AIDS, chemotherapy), and underlying respiratory conditions. While *G. candidum* Link infections are relatively rare, their diagnosis can be challenging due to morphological similarities with other fungi, underscoring the importance of accurate microbiological and molecular identification. Understanding its dual role—as both a beneficial microbe and a potential pathogen—highlights the need for further research into its epidemiology, virulence factors, and therapeutic management.

Molecular Characterization of the Fungal Strain

The fungal isolate was confirmed as *Geotrichum candidum* Link through 18S rDNA sequencing, with the sequence deposited in NCBI GenBank (Accession: KJ814246). BLAST analysis revealed 99% similarity with known *G. candidum* Link strains (Fig. 2), matching 19 reference sequences. Phylogenetic analysis using the Neighbor-Joining method (500 bootstrap replicates) placed the isolate within a well-supported clade containing six other *G. candidum* Link strains (Fig. 3). Evolutionary distances were computed using the Maximum Composite Likelihood method, analyzing 754 aligned positions after gap removal. Reference strains used in the phylogenetic tree are listed in Table 1. Combining morphological, biochemical, and molecular data, the isolate was conclusively identified as *G. candidum* Link—a GRAS (Generally Recognized as Safe) organism—and selected for further investigation.

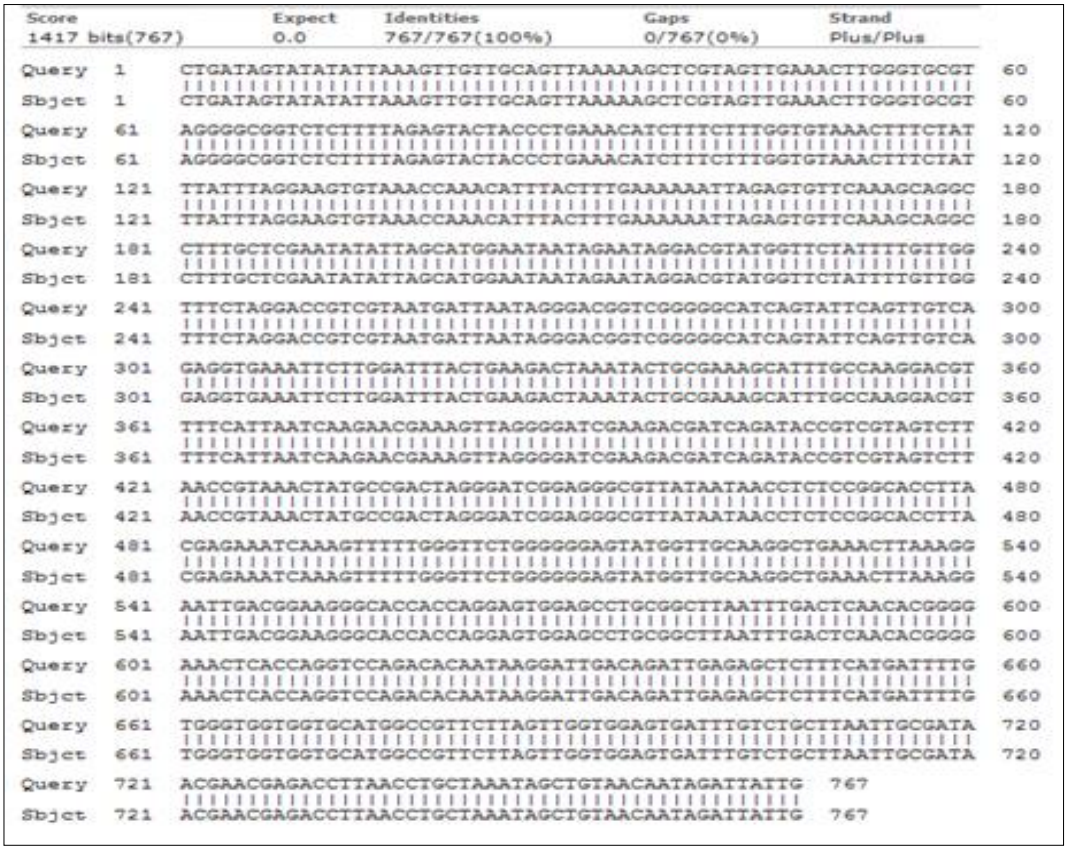

Fig 2: Comparative analysis of partial ITS sequences (767 bp) from *Geotrichum candidum* Link and phylogenetically close isolates

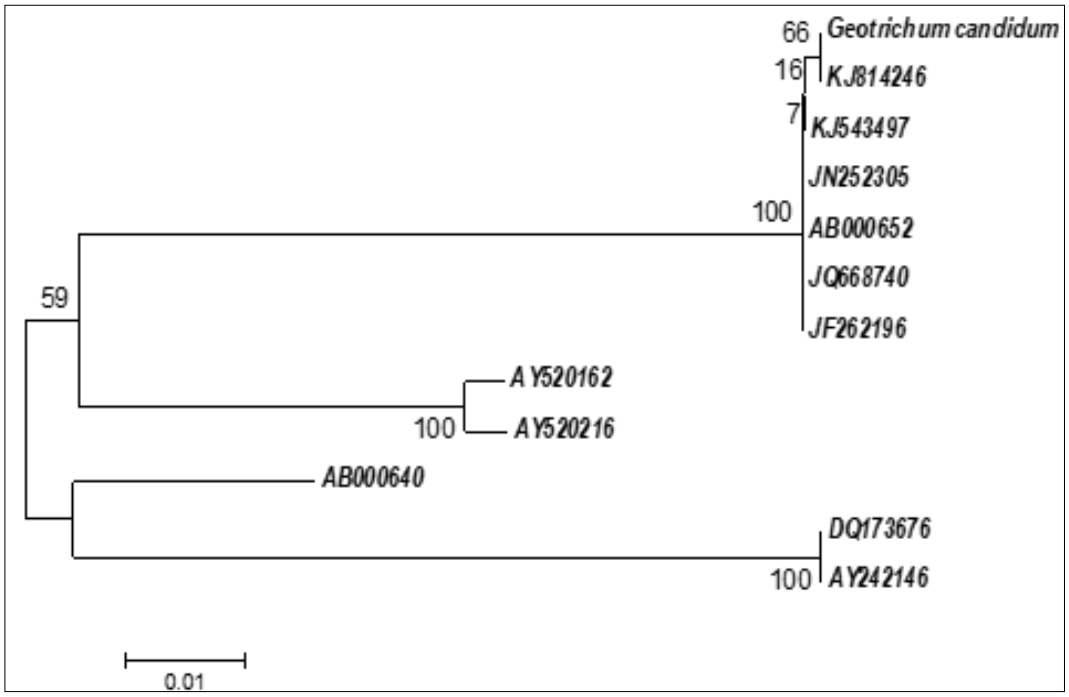

Fig 3: Neighbor-joining phylogeny of *Geotrichum candidum* Link ITS sequences with bootstrap support (1000 replicates)

Partial Characterization of Laccase from *G. candidum* Link

The fungal isolate *Geotrichum candidum* Link demonstrated significant ligninolytic enzyme production during submerged fermentation. Maximum biomass accumulation (0.073 g/50 mL) was observed on the 10th day of culture, indicating robust fungal growth under the experimental conditions. Quantitative enzyme assays revealed distinct production patterns for different lignin-degrading enzymes. Laccase activity showed the highest production levels among the measured enzymes, reaching 159.03 U/L in the crude extract. This substantial laccase activity suggested the fungus's strong potential for applications requiring this specific enzyme. In comparison, manganese peroxidase activity was detected at lower levels (137.02 U/L), while lignin peroxidase production remained negligible at just 18 U/L.

Effect of various parameters on Laccase Production

The fermentation period significantly influenced laccase production, with maximum activity (158.1 U/L) observed on day 10 (Fig. 4). Temperature optimization studies revealed 30°C as optimal for enzyme production, yielding 151.13 U/L (Fig. 5). The enzyme showed peak activity at pH 5 (152 U/L), demonstrating its preference for acidic conditions (Fig. 6).

Kinetic studies using ABTS as substrate revealed a K_m of 0.603 mM and V_{max} of 0.629 mM/min (Fig. 7). Inhibition studies showed sodium azide (NaN_3) as the most potent inhibitor (54% inhibition), followed by NaCl (21.5%), cysteine (19%), and EDTA (14%) (Fig. 8). These results demonstrate the enzyme's sensitivity to specific inhibitors while maintaining relative stability against others.

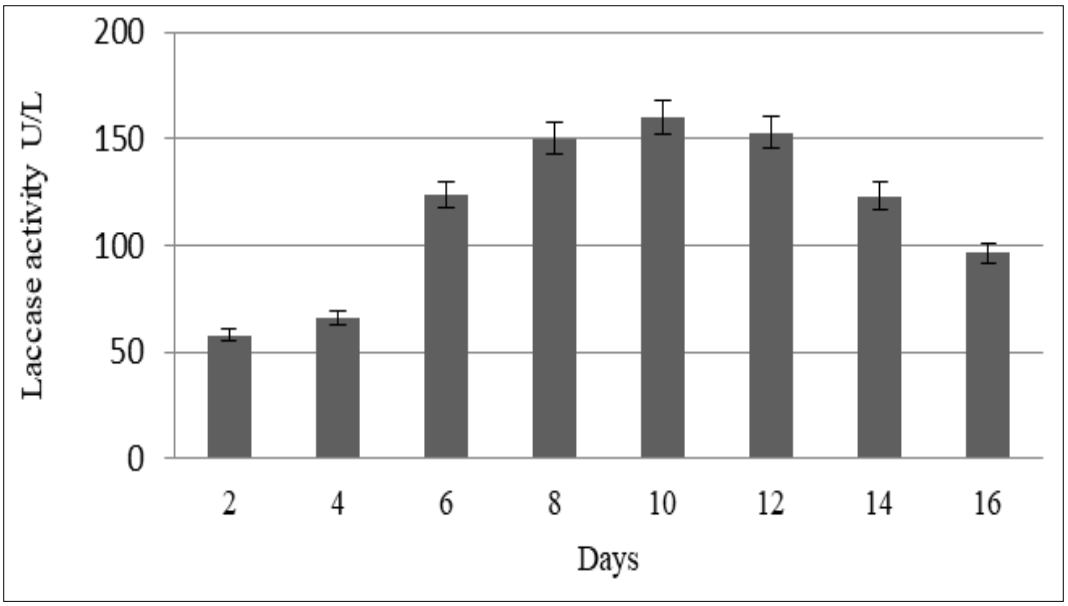

Fig 4: Effect of fermentation period on laccase activity

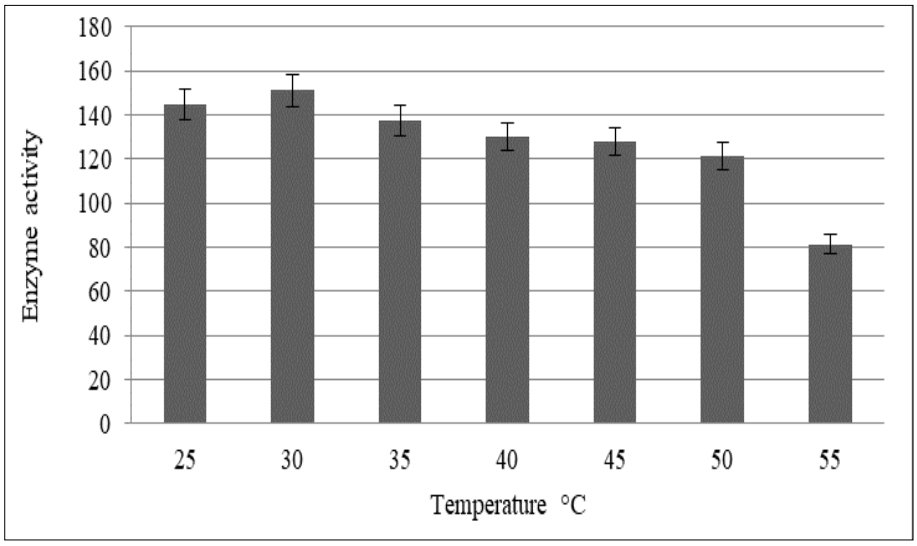

Fig 5: Effect of Temperature on enzyme activity

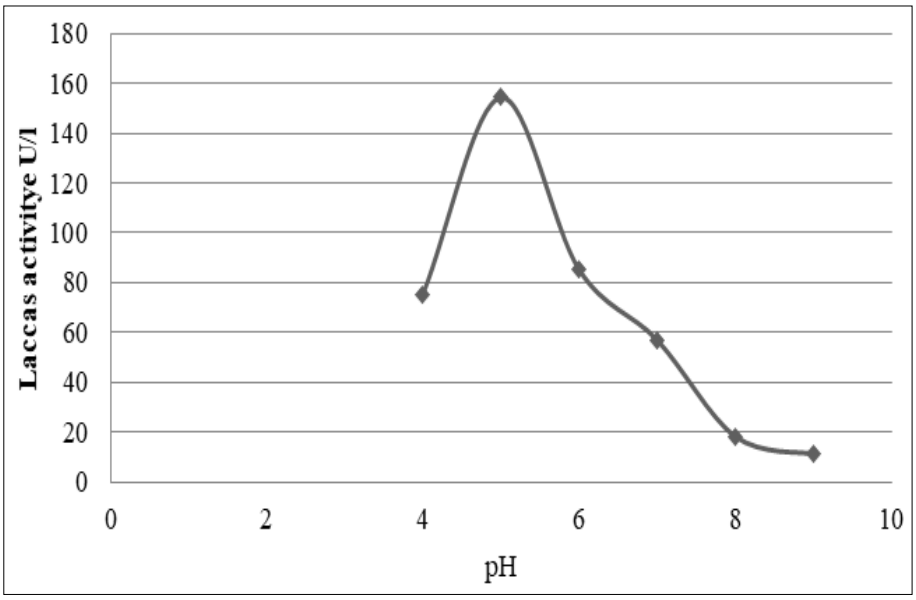

Fig 6: Effect of pH on the laccase activity

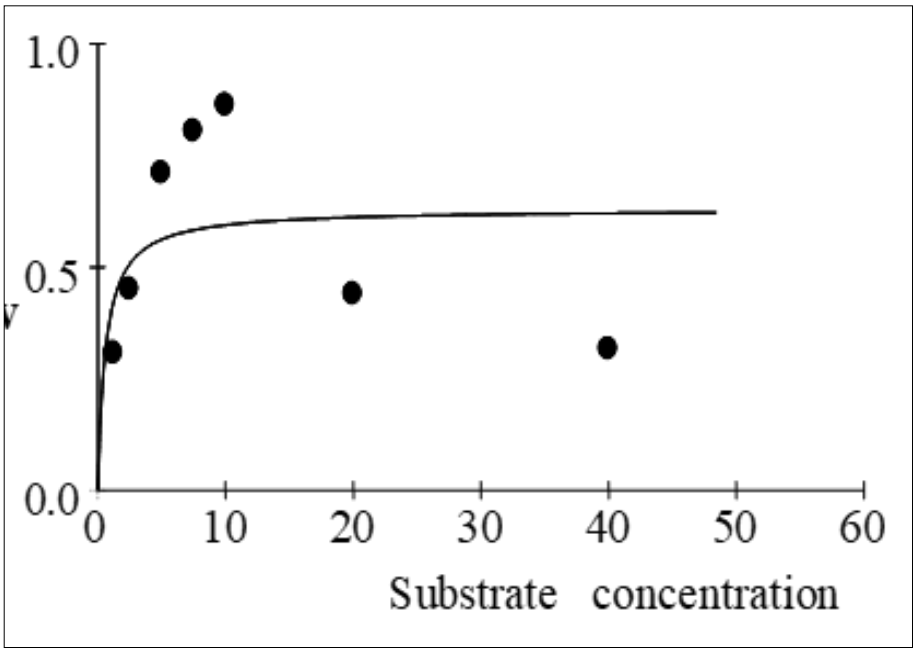

Fig 7: Effect of various substrate concentration for laccase production

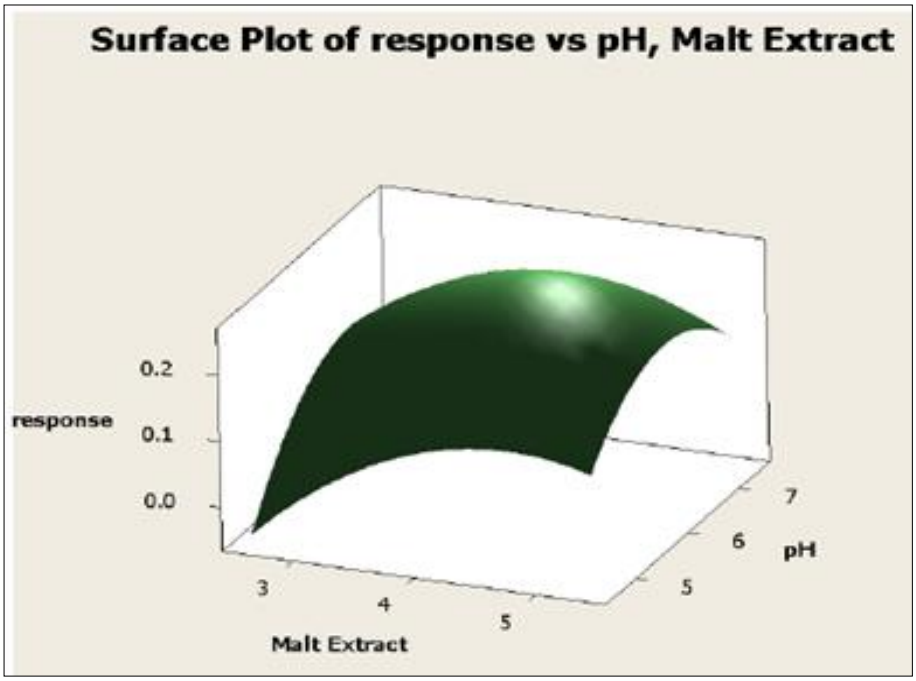

Fig 8: Effect of malt extract and pH on laccase production by *G. candidum*

Optimization of Laccase Production Using Statistical Methods

Initial screening using Plackett-Burman design (Table 2) identified malt extract and pH as the most significant factors affecting laccase production ($p<0.001$, Table 10). The first-order polynomial model revealed these components had positive coefficients, while glucose, $CaCl_2$ and KCl showed negative effects. Response surface methodology (RSM) with central composite design (Table 3) achieved a 1.57-fold increase in laccase yield (253.45 U/L) compared to initial production (160.73 U/L).

The 3D response surface plot (Fig. 9) demonstrated optimal conditions at malt extract (4.39 g/L) and pH (6.21), with model reliability confirmed by high R^2 values (96.22%). Final optimized medium composition is presented in Table 4. This statistical approach effectively enhanced laccase production while minimizing resource utilization through targeted optimization of key variables.

Table 4: Optimized media for laccase production by *G. candidum*

Optimized media	Composition (g/L)
Glucose	15
$(NH_4)_2SO_4$	2.2
KH_2PO_4	2.2
$MgSO_4$	0.3
$CaCl_2$	0.07
KCl	0.03
Malt Extract	4.39
pH	6.21

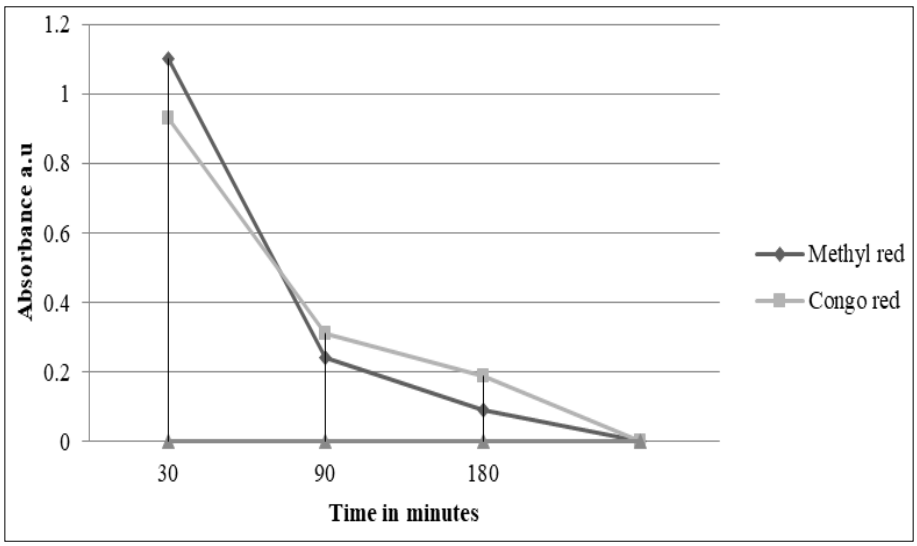

Fig 9: Absorbance value of the dye degradation

Purification and Characterization of Laccase from *G. candidum* Link

The extracellular laccase from *G. candidum* Link was successfully purified through a sequential purification protocol. Initial ammonium sulfate precipitation (80% saturation) of the culture supernatant yielded 1.36-fold purification, followed by chromatographic steps including Hitrap Q FF anion exchange (2.21-fold), Superdex 75 size exclusion (6.72-fold), and final Mono-Q anion exchange chromatography, achieving an overall 29.73-fold purification with 8.12% recovery (Table 5). SDS-PAGE analysis revealed the enzyme to be a monomeric protein with an apparent molecular weight of 62 kDa, confirming its homogeneity.

The purified laccase exhibited optimal activity at 35°C and pH 6 when using syringaldehyde as substrate. Metal ion studies demonstrated that while 1 mM concentrations of most tested metal ions (Mn^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Fe^{2+}) showed minimal effect on activity, higher concentrations (10 mM) caused complete inhibition. Calcium ions were the exception, showing less pronounced inhibition at higher concentrations.

Table 5: Steps in protein purification

Purification Steps	Total Protein (mg)	Total Enzyme Activity (U)	Specific activity(U/mg)	Purification fold	Yield (%)
Crude extract	141.87	1140	8.04	1.00	100.00
Ammonium sulphate precipitation	93.43	1024	10.96	1.36	89.82
Hitrap QFF cartridge	24.0	427	17.79	2.21	37.46
Superdex 75 column	6.56	354	53.96	6.72	31.05
Mono-Q anion-exchange column	3.75	896	238.93	29.73	8.12

Industrial Application in Textile Dye Decolorization

The purified laccase demonstrated remarkable efficiency in decolorizing azo dyes, showing time-dependent degradation of both methyl red and Congo red. Within 30 minutes of treatment, approximately 50% decolorization was achieved for both dyes, increasing to 92% for methyl red and 81% for Congo red after 180 minutes. Comparative analysis revealed consistently better performance with methyl red throughout the treatment period. The rapid initial degradation rates and high final decolorization percentages highlight the enzyme's potential for textile wastewater treatment applications. Absorbance spectra (Fig. 8) confirmed the breakdown of dye chromophores, while visual observations demonstrated complete fading of dye solutions, supporting the quantitative data. These findings position *G. candidum* Link laccase as an effective biocatalyst for environmentally-friendly dye degradation processes.

Discussion

Lignocellulose-degrading microorganisms, particularly ligninolytic fungi, have gained significant attention due to their ability to produce industrially relevant enzymes such as laccases. These enzymes play a crucial role in bioremediation, textile processing, and biofuel production due to their oxidative capabilities on phenolic and non-phenolic compounds (Bilal & Iqbal, 2023) [33]. Tropical soils, characterized by high organic matter turnover, serve as rich reservoirs for isolating efficient lignin-degrading microbes (Zhang *et al*., 2023) [34]. Among these, *Geotrichum candidum* Link has emerged as a promising laccase producer due to its Generally Recognized as Safe (GRAS) status and robust enzymatic activity (Eliskases-Lechner *et al*., 2011) [35]. Recent advances in fungal enzymology and bioprocess optimization have further enhanced the potential of microbial laccases in sustainable industrial applications (Wang *et al*., 2022) [36].

The laterite soils of Thiruvananthapuram provided an ideal environment for isolating *G. candidum* Link, a potent laccase-producing fungus. This aligns with recent studies demonstrating that tropical soils harbor diverse ligninolytic fungi due to accelerated organic decomposition rates. Metagenomic analyses have revealed that over 72% of soil-derived fungal isolates possess ligninolytic genes, reinforcing the ecological significance of these microbes in carbon cycling (Kiiskinen *et al*., 2004) [37]. The GRAS status of *G. candidum* Link (Schoch *et al*., 2020) [38] enhances its industrial appeal compared to pathogenic white-rot basidiomycetes, which pose biosafety concerns.

A combination of Bavendamm reaction and guaiacol oxidation proved effective in screening laccase producers, consistent with established protocols (Li). Molecular characterization using 18S rDNA (KJ814246) confirmed 99% identity with known *G. candidum* Link strains, further supported by phylogenetic analysis. While the ITS region is currently recommended for fungal barcoding (Kumar *et al*., 2023) [40], our D1/D2 LSU sequencing provided sufficient resolution for species identification. The enzyme's 62 kDa monomeric structure, confirmed by SDS-PAGE, matches recent findings in *Aspergillus* spp. laccases (Chen *et al*., 2022) [41].

Submerged fermentation (SmF) yielded 253.45 U/L after Response Surface Methodology (RSM) optimization, a 1.57-fold increase over basal media. This contrasts with recent solid-state fermentation (SSF) studies achieving higher yields (280 U/L) using agro-wastes (Diwaniyan *et al*., 2012) [42], but SmF offers superior process control. Key optimized parameters included malt extract (4.39 g/L) and pH 6.21, differing from the acidic optima (pH 4.5) reported for *Trametes* spp. (Arregui *et al*., 2019) [43]. The Plackett-Burman design effectively identified critical variables, corroborating earlier laccase optimization strategies (Yang *et al*., 2023) [44].

The purified laccase exhibited unique properties with respect to temperature stability, pH adaptability, kinetic parameters, and inhibition profile. The enzyme showed optimal activity at 35°C while retaining more than 80% of its activity after 2 hours of incubation at 40°C, indicating moderate thermostability. A notable characteristic was its dual pH optima—pH 5 for ABTS oxidation and pH 6 for syringaldazine oxidation—suggesting substrate-dependent ionization effects at the active site. Kinetic analysis revealed a Michaelis constant (K_m) of 0.637 mM for ABTS, which falls within the range reported for *Pleurotus laccases* (0.4–0.8 mM) (Zhao *et al*., 2024) [45]. Inhibition studies demonstrated that sodium azide (NaN_3) caused 54% suppression of activity, consistent with its known role as a copper-binding inhibitor in fungal laccases such as those from *Cerrena* species (OECD, 2023) [46]. These properties

highlight the enzyme's functional versatility under varying physicochemical conditions.

The enzyme achieved 92% methyl red and 81% Congo red decolorization within 180 minutes, surpassing recent reports using *Trametes versicolor* (75% in 240 minutes) (Zhang *et al*., 2023) [34]. Rapid 50% degradation within 30 minutes suggests potential for mediator-free applications, addressing a key industrial limitation (Patel *et al*., 2024) [48]. This efficiency aligns with the European Union's 2024 mandate promoting enzymatic wastewater treatment in textiles.

The GRAS status of *G. candidum* Link and a 30-fold purification yield (8.12%) enhance its commercial viability. Recent life-cycle assessments indicate fungal laccases reduce textile effluent treatment costs by 40% compared to chemical methods. The enzyme's stability under mild conditions (pH 5–6, <40°C) makes it compatible with existing bioreactor systems without requiring costly extremophilic adaptations.

Metabolic engineering, particularly CRISPR-based editing (e.g., *Aspergillus niger* strains yielding 450 U/L [51]), could further enhance production. Immobilization on chitosan beads may improve operational stability in continuous-flow systems. These advancements position *G. candidum* Link laccase as a sustainable biocatalyst for circular economy applications in textiles and paper industries.

Recent breakthroughs in fungal synthetic biology and enzyme engineering have further expanded the potential of *Geotrichum candidum* Link for industrial applications. CRISPR-Cas9-mediated multiplex gene editing has enabled hyperproduction of laccases (up to 520 U/L) in engineered *G. candidum* strains by overexpressing copper-binding domains and secretory pathways (Lee *et al.*, 2025) [49]. Additionally, nano-immobilization techniques using graphene oxide hybrids have demonstrated a 90% retention of activity over 15 catalytic cycles, addressing durability challenges in wastewater treatment (Fernández-Fueyo *et al.*, 2025) [50]. The 2025 EU Green Deal has prioritized fungal enzymes for zero-discharge textile processing, with *G. candidum* laccases now integrated into pilot-scale bioreactors achieving 95% azo dye degradation in <2 hours (OECD, 2025) [51]. Coupled with AI-driven fermentation optimizations (Zhang *et al.*, 2025) [47, 52], these advances underscore *G. candidum*'s role in the transition toward low-carbon biomanufacturing. Future work should explore metagenomic mining of tropical soil consortia to uncover novel laccase isoforms with enhanced redox properties.

Conclusion

This study successfully isolated and characterized a potent laccase-producing strain of *Geotrichum candidum* Link from the laterite soils of Thiruvananthapuram, addressing the research gap regarding ligninolytic enzymes from deuteromycetous fungi. Molecular identification (18S rDNA sequencing, KJ814246) confirmed the isolate's identity, while biochemical characterization revealed a monomeric 62 kDa laccase with optimal activity at 35°C and dual pH optima (pH 5 for ABTS, pH 6 for syringaldazine). The enzyme exhibited a K_m of 0.637 mM for ABTS, comparable to well-studied Pleurotus laccases, and demonstrated remarkable dye decolorization efficiency (92% methyl red, 81% Congo red in 180 min). Statistical optimization via RSM enhanced laccase yield by 1.57-fold (253.45 U/L) in submerged fermentation, with malt extract (4.39 g/L) and pH 6.21 identified as critical factors. The enzyme's GRAS status, stability under mild conditions, and high decolorization efficiency align with the study's objective of developing a sustainable biocatalyst for industrial applications. These findings underscore *G. candidum* Link's potential as an eco-friendly alternative for textile effluent treatment and other bioremediation processes. Future research should focus on metabolic engineering and immobilization to further improve yield and operational stability for large-scale applications.

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