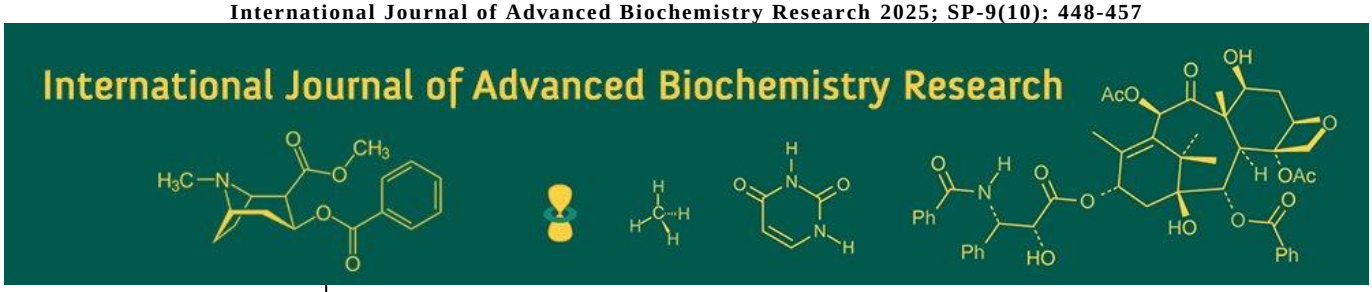

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(10): 448-457
www.biochemjournal.com
Received: 11-07-2025
Accepted: 15-08-2025

MB Vala
N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

RP Bambharolia
N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

AJ Deshmukh
College of Agriculture, Navsari Agricultural University, Waghai, Gujarat, India

NH Garaniya
College of Agriculture, Navsari Agricultural University, Bharuch, Gujarat, India

DM Damasia
College of Agriculture, Navsari Agricultural University, Waghai, Gujarat, India

SG Patel
College of Agriculture, Navsari Agricultural University, Waghai, Gujarat, India

NM Thesiya
N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

MD Khunt
N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

PA Vavdiya
Hill Millet Research Station, Navsari Agricultural University, Waghai, Gujarat, India

Corresponding Author:
RP Bambharolia
N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

Lignin degradation potential of *Staphylococcus cohnii* MV19 isolated from the Dangs region, Gujarat

MB Vala, RP Bambharolia, AJ Deshmukh, NH Garaniya, DM Damasia, SG Patel, NM Thesiya, MD Khunt and PA Vavdiya

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10Sf.5872

Abstract
The present study reports the isolation and characterization of lignin degrading bacteria from diverse lignin rich habitats of the Dangs region, Gujarat. A total of 25 bacterial isolates were obtained through enrichment on wheat straw based mineral salt medium (MSM), of which four (MV11, MV17, MV19 and MV22) consistently exhibited ligninolytic activity on ABTS, o-dianisidine, guaiacol and Azure B plates. Among these, isolate MV19 showed the highest potency index and was identified as *Staphylococcus cohnii* through 16S rRNA sequencing (GenBank Accession No. PV876755). Enzymatic assays confirmed the simultaneous production of laccase, manganese peroxidase and lignin peroxidase, highlighting broad ligninolytic capacity. Optimization under submerged fermentation revealed glucose (1%) and urea (0.25%) as the most suitable carbon and nitrogen sources, while pH 6.0 and 30±2 °C favored maximum enzyme secretion. Under these conditions, MV19 achieved a peak laccase activity of 26.74 U/ml at 96 hrs, accompanied by high specific activity and protein yield. The findings establish *S. cohnii* MV19 as a promising ligninolytic bacterium with potential applications in biobleaching, lignocellulosic biomass valorization, wastewater treatment and environmental bioremediation.

Keywords: ABTS, *Staphylococcus cohnii*, o-dianisidine, Azure B, laccase, lignin peroxidase (LiP), manganese peroxidase (MnP)

1. Introduction
Lignin is a structurally complex, high molecular weight aromatic polymer, second only to cellulose in abundance. Its irregular, three dimensional network, formed from p-coumaryl, coniferyl, and sinapyl alcohols, provides rigidity, hydrophobicity, and pathogen resistance in plant secondary cell walls but makes lignin highly recalcitrant to chemical and enzymatic degradation (Palmqvist and Hagerdal, 2000; Perez *et al.*, 2002; Pollegioni *et al.*, 2015) [23, 24, 26]. This recalcitrance poses challenges for industrial processing of lignocellulosic biomass and biofuel production.

A limited number of microorganisms, including fungi, actinomycetes, and bacteria, can degrade lignin through extracellular lignin-modifying enzymes (LMEs) such as laccases, lignin peroxidases (LiP), and manganese peroxidases (MnP) (Bohacz and Kowalska, 2020; Li *et al.*, 2020; and Adarsh and Chandra, 2020) [6, 17, 2]. Among these, bacterial LMEs are of particular interest due to their faster growth rates, environmental tolerance, smaller enzyme size, and potential for genetic manipulation (Chandra *et al.*, 2015 and Tian *et al.*, 2016) [9, 38]. Bacterial genera such as *Paenibacillus, Bacillus, Pseudomonas,* and *Streptomyces* have demonstrated the ability to depolymerize lignin by cleaving ether and carbon carbon bonds within the lignin matrix (Ball *et al.*, 1989; Ghosh *et al.*, 2021 and Utarti *et al.*, 2024) [4, 12, 39]. These microbes secrete oxidoreductive enzymes that generate phenoxy radicals, facilitating lignin depolymerization and enabling utilization of lignin derived oligomers for microbial metabolism.

Ligninolytic enzymes have extensive industrial applications. Laccases and peroxidases are used in pulp and paper bleaching, dye degradation, soil and wastewater bioremediation, biosensor development, and the bioconversion of lignocellulosic waste into value added products such as vanillin and biodegradable plastics (Yadav and Yadav, 2015 and Siva *et al.*, 2021) [41, 33]. Bacterial enzymes are often more thermostable, alkaline tolerant, and mediator independent than their fungal counterparts, making them attractive for industrial processes.

From an ecological perspective, bacterial ligninolysis accelerates the turnover of recalcitrant carbon in soils, contributing to nutrient recycling and long term carbon sequestration. The presence of lignin degrading bacteria in forest litter, compost, and agro-industrial waste highlights their ecological significance and potential for sustainable biomass management (Campbell and Sederoff, 1996 and Chandra *et al.*, 2015) [8, 9]. Considering the ecological and industrial significance of ligninolytic bacteria, this study aims to isolate and characterize lignin degrading bacteria from the Dangs region of Gujarat.

2. Materials and Methods

2.1 Enrichment, isolation and characterization of ligninolytic bacteria

2.1.1 Collection of soil sample and isolation of bacteria

For isolation of lignin degrading bacteria, samples were collected from decomposed manures (Dodipada, Waghai), forest litter (Waghai), city wastes (borpada), farmyard manure (Cafeteria, CoA, Waghai), forest soil (Waghai), forest degraded wood (Waghai), farmyard manure (Rajendrapur Farm) and rural compost (Waghai) *etc*. approximately 20 g of the sample were collected in the sterile polythene bag and taken into the laboratory for further analysis.

2.1.2 Isolation of lignin degrading bacteria

Lignin degrading bacteria were isolated by the enrichment method. For enrichment 1.0 g of each collected sample was added separately in mineral salt medium (MSM) broth containing 1.0% of wheat straw as the sole carbon source and incubated at 35±2 °C for 20 days under the shaking condition. From this 1.0 ml of sample was serially diluted up to 10^{-6}, and then the 10^{-4}, 10^{-5} and 10^{-6} dilutions were plated onto the Lignin Basal Medium (LBM) (Archibald, 1992) [3] (g/l of KH_2PO_4, 1.0; $C_4H_{12}N_2O_6$, 0.5; $MgSO_4.7H_2O$, 0.5; $CaCl_2.2H_2O$, 0.01; Yeast Extract, 0.01; $CuSO_4.H_2O$, 0.001; $Fe_2(SO_4)_3$, 0.001; $MnSO_4.H_2O$, 0.001; Agar, 16.0) supplemented with 0.1% w/v ABTS (2,2-Azino bis (3-ethylbenz-thiazoline-6-sulfonic acid)) and separately sterilized 1 ml of 20% w/v aqueous glucose for each 100 ml of growth medium. Plates were incubated at 35±2 °C and examined periodically for the formation of a green color zone around colonies. Colonies that showed green color was purified by repeated streaking. The purified colonies were preserved at 4 °C on Nutrient Agar (NA) for further studies.

2.1.3 Morphological and biochemical characterization of bacteria

Bacterial isolates were characterized based on colony morphology (size, shape, margin, texture, opacity, pigmentation) and cellular features such as cell size, shape, arrangement and Gram's reaction. Biochemical profiling was performed using the Hi-Assorted biochemical test kit (HiMedia, Mumbai, India), which includes twelve conventional tests (Indole, Methyl Red, Voges-Proskauer, Citrate utilization and fermentation of glucose, adonitol, arabinose, lactose, sorbitol, mannitol, rhamnose and sucrose). In addition, standard assays such as starch hydrolysis, nitrate reduction, urease activity, phenylalanine deamination, gelatin hydrolysis, H_2S production, motility, catalase reaction and acid/gas production from various sugars were carried out. Results were interpreted according to manufacturer guidelines based on colorimetric changes after incubation at 37 °C for 24 hrs.

2.2 Screening of lignin degrading bacteria for ligninolytic enzymes in plates

2.2.1 Screening of lignin degrading bacteria for ligninolytic enzymes in plates

Isolated bacterial strains were subjected to screening for extracellular ligninolytic enzymes, including laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP), using qualitative plate based assays. For laccase activity, isolates were spot inoculated on ABTS/o-dianisidine agar, where the development of a characteristic color change indicated positive enzyme production (Archibald, 1992) [3]. MnP activity was assessed on basal salts agar supplemented with guaiacol, the MnP producing strains were confirmed by a halo zone formation around the colony by the oxidation of guaiacol (Rogalski *et al.*, 1991) [31]. LiP activity was detected on Azure B agar plates, where enzymatic degradation was confirmed by dye decolorization surrounding the colonies, reflecting lignin peroxidase secretion (Archibald, 1992) [3]. Together, these assays provided an efficient and rapid means of identifying potential ligninolytic bacteria for further characterization.

2.2.2 Potency index

The relative potency of laccase producing isolates was evaluated on ABTS agar (LBM supplemented with 0.1% w/v ABTS and glucose). Oxidation of ABTS to ABTS-azine resulted in green zone formation around colonies (Niku-Paavola *et al.*, 1990) [21]. The potency index (PI) was calculated as the ratio of zone diameter to colony diameter, providing a comparative measure of laccase activity (Teck *et al.*, 2011). [37]

2.2.3 Identification of potential lignolytic isolates

The most potent ligninolytic isolate was identified through 16S rRNA gene sequencing (Petti, 2007) [25]. Genomic DNA was extracted using the CTAB method, quality-checked on agarose gel, and amplified with universal eubacterial primer 27F. The purified PCR product was sequenced (ABI 3730xl Genetic Analyzer, Saffron Life Sciences, Surat), and homology was determined using BLAST against NCBI databases. Phylogenetic analysis was performed, and the sequence was deposited in GenBank.

2.3 Optimization of different physico-chemical parameters for laccase production under submerged conditions

2.3.1 Optimization of laccase production

Liquid media optimization of the most potent isolate (MV19) was carried out using a one factor at a time (OFAT) approach, where individual parameters were varied while others were kept constant (Tandel, 2013) [36]. Bushnell and Hass medium (BHM) served as the basal medium to assess the influence of different carbon sources (glucose, lactose, maltose, starch, sucrose, fructose, xylan; 1% w/v), nitrogen sources (peptone, urea, ammonium tartrate, gelatin; 0.25% w/v), incubation temperatures (20 to 50 °C) and pH values (5 to 9) on growth and laccase production. Cultures were incubated at 35±2 °C and 120 rpm for up to 120 hrs, with enzyme secretion monitored at 24 hrs intervals. Supernatants obtained after centrifugation (6,000 rpm, 20 min, 4 °C) were assayed for laccase activity.

2.3.2 Laccase activity
Laccase activity was determined from crude enzyme extracts obtained by centrifugation of culture broth (6000 rpm, 20 min, 4 °C). Enzyme activity was assayed by monitoring ABTS oxidation at 420 nm (ε420 = 36,000 M^{-1} cm^{-1}) in a reaction mixture containing sodium acetate buffer (pH 4.5), ABTS and enzyme solution at 35±2 °C (Wolfenden and Wilson, 1982) [40]. One unit of laccase activity was defined as the amount of enzyme catalyzing the oxidation of 1 µM ABTS per min under assay conditions.

2.3.3 Protein analysis
Crude protein content from culture supernatant (centrifuged at 6000 rpm, 20 min, 4 °C) was estimated by the Folin Lowry method using Bovine Serum Albumin (BSA) as standard (Lowry *et al.*, 1951) [18]. The assay is based on the reaction of Folin-Ciocalteau reagent with tyrosine and tryptophan residues, producing a bluish-purple complex measured at 660 nm.

3. Results and Discussion
3.1 Isolation, screening, characterization and identification of lignin degrading bacteria
3.1.1 Isolation and screening of lignin degrading bacteria
A total 25 (MV1 to MV25) bacterial isolates with varied colonical characters such as size, shape, margin, elevation, texture, pigmentation, *etc.* were obtained from collected samples using enrichment enrichment based isolation approaches (Table 1 and 2). Similar enrichment based isolation approaches were reported by Sasikumar *et al.* (2014) [32]; Keerthana *et al.* (2019) [16] and Sumranwanich *et al.* (2024) [34]. Most isolates were medium sized with round or irregular forms, smooth surfaces and either viscous or butyrous consistency. Elevation types varied from pulvinate and raised to flat and convex. Pigmentation was predominantly white, with a few isolates displaying yellowish or fuzzy appearances. Opacity ranged from opaque to nacreous and opalescent. These diverse colonical features aid in the preliminary differentiation of the bacterial isolates. Isolated bacteria were screened for lignolytic activity on o-dianisidine and ABTS agar. Bacteria with lignolytic activity changed the color around the colonies on o-dianisidine agar and the appearance of green color around the colonies on ABTS agar was considered as positive for lignolytic activities. Out of the total 25 bacterial isolates, 10 isolates (MV1, MV3, MV4, MV5, MV9, MV10, MV11, MV17, MV19 and MV22) showed color change around the colonies on o-dianisidine agar and 7 isolates (MV2, MV4, MV11, MV14, MV17, MV19, MV22) showed green color zone around the colonies on ABTS agar (Table 3). While, bacterial isolates MV11, MV17, MV19 and MV22 were showed positive reaction on both agar (o-dianisidine and ABTS agar) and selected for further studies. Likewise, Dhouib *et al.* (2005) [10]; Huang *et al.* (2013) [14] and Siva *et al.* (2021) [33] used ABTS supplemented plates for primary screening of laccase producing microorganisms.

Table 1: Number of bacteria isolated from different samples of Dangs

Sr. No.	Sampling site	Number of bacteria isolated	Code of Bacterial Isolates
1	Decomposed manures, Dodipada, Waghai	5	MV1
			MV2
			MV3
			MV4
			MV5
2	Forest litter, Waghai	3	MV6
			MV7
			MV8
3	City wastes, Borpada, Waghai	1	MV9
4	Farmyard manure, Cafeteria, CoA, Waghai	5	MV10
			MV11
			MV12
			MV13
			MV14
5	Forest soil, Waghai	1	MV15
6	Forest degraded wood, Waghai	5	MV16
			MV17
			MV18
			MV19
			MV20
7	Farmyard manure, Rajendrapur Farm, Waghai	5	MV21
			MV22
			MV23
			MV24
			MV25

Table 2: Colonical characters of bacterial isolates

Isolates	Size	Form	Margin	Elevation	Surface	Consistency	Pigmentation	Opacity
MV1	Medium	Round	Entire	Pulvinate	Smooth	Viscous	Fuzzy white	Opaque
MV2	Small	Round	Undulate	Umbonate	Smooth	Butyrous	White	Opaque
MV3	Small	Punctiform	Entire	Convex	Smooth	Viscous	White	Opalescent
MV4	Small	Round	Entire	Umbonate	Smooth	Viscous	White	Nacreous
MV5	Large	Conglomerate	Erose	Raised	Smooth	Butyrous	White	Opaque
MV6	Medium	Irregular	Undulate	Raised	Wrinkled	Viscous	White	Opaque
MV7	Medium	Round	Entire	Pulvinate	Smooth	Viscous	Yellowish white	Nacreous
MV8	Medium	Conglomerate	Entire	Convex	Smooth	Viscous	White	Opaque
MV9	Small	Punctiform	Entire	Pulvinate	Smooth	Butyrous	White	Opaque
MV10	Large	Irregular	Undulate	Raised	Rough	Butyrous	White	Opaque
MV11	Medium	Irregular	Undulate	Flat	Rough	Dry	White	Cretaceous
MV12	Medium	Round	Entire	Pulvinate	Smooth	Viscous	White	Opaque
MV13	Medium	Conglomerate	Entire	Convex	Smooth	Viscous	White	Nacreous
MV14	Medium	Conglomerate	Entire	Raised	Rough	Viscous	White	Opaque
MV15	Medium	Round	Entire	Conical	Smooth	Butyrous	White	Opaque
MV16	Small	Punctiform	Entire	Flat	Smooth	Butyrous	White	Opalescent
MV17	Medium	Irregular	Lobate	Flat	Rough	Powdery	White	Opaque
MV18	Small	Punctiform	Entire	Pulvinate	Smooth	Dew drop	Yellow	Nacreous
MV19	Medium	Round	Entire	Raised	Smooth	Butyrous	Pale yellow	Opaque
MV20	Small	Punctiform	Entire	Raised	Smooth	Dew drop	White	Nacreous
MV21	Medium	Conglomerate	Entire	Pulvinate	Smooth	Butyrous	White	Opaque
MV22	Medium	Round	Entire	Flat	Rough	Dry	White	Opaque
MV23	Medium	Round	Entire	Convex	Smooth	Dew drop	White	Cretaceous
MV24	Medium	Round	Undulate	Pulvinate	Smooth	Viscous	White	Opaque
MV25	Medium	Round	Entire	Pulvinate	Smooth	Viscous	White	Opaque

Table 3: Screening of bacterial isolates on different agar

Sr. No.	Code of Bacterial Isolates	Result on o-dianisidine agar	Result on ABTS agar
1	MV1	+	-
2	MV2	-	+
3	MV3	+	-
4	MV4	+	+ +
5	MV5	+	-
6	MV6	-	-
7	MV7	-	-
8	MV8	-	-
9	MV9	+	-
10	MV10	+	-
11	MV11	+ +	+ + +
12	MV12	-	-
13	MV13	-	-
14	MV14	-	+ +
15	MV15	-	-
16	MV16	-	-
17	MV17	+ +	+ + +
18	MV18	-	-
19	MV19	+ + +	+ + +
20	MV20	-	-
21	MV21	-	-
22	MV22	+ +	+ +
23	MV23	-	-
24	MV24	-	-
25	MV25	-	-

3.1.2 Morphological and biochemical characterization of potent lignin degrading bacteria

Four lignin degrading bacterial isolates (MV11, MV17, MV19, and MV22) were characterized morphologically and biochemically (Table 4 and 5). Isolate MV11 was a motile, Gram-negative rod, while MV17 was a Gram-positive oval shaped bacterium arranged in chains with motility. MV19 showed Gram positive, oval shaped cells in staphylococcal clusters but lacked motility. MV22 was Gram positive, rod shaped, with chain like arrangements and motile. The predominance of Gram positive isolates is in agreement with earlier reports highlighting as dominant ligninolytic groups (Bugg *et al*., 2011; Ghosh *et al*., 2021) [7, 12].

Table 4: Morphological characters of potent lignin degrading isolates

Potent isolates	Cell shape	Cell arrangement	Gram's reaction	Motility
MV11	Rod	Single	Negative	Motile
MV17	Oval	Chain	Positive	Motile
MV19	Oval	Staphylococci	Positive	Non-motile
MV22	Rod	Chain	Positive	Motile

Table 5: Biochemical characters of potent lignin degrading isolates

Biochemical tests	MV11	MV17	MV19	MV22
Indole production	+	+	-	+
Methyl red	+	+	+	+
Voges-Proskauer	+	+	+	+
Citrate utilization	+	+	+	+
Starch hydrolysis	+	+	+	+
Nitrate reduction	+	+	+	+
Urease activity	-	-	+	-
Phenylalanine deaminase	+	-	-	+
Gelatin hydrolysis	+	+	+	+
H_2S production	-	-	-	-
Motility	+	+	-	+
Catalase	+	+	+	+
Acid and gas production from different sugars				
Glucose	+	+	+	+
Adonitol	-	-	-	-
Arabinose	+	-	-	-
Lactose	-	+	-	-
Sorbitol	-	-	-	+
Mannitol	-	-	-	-
Rhamnose	+	-	-	-
Sucrose	+	+	+	+

Biochemical tests revealed diverse metabolic capabilities. All isolates tested positive for MR, VP, citrate utilization, starch hydrolysis, nitrate reduction, gelatin hydrolysis, catalase activity, and carbohydrate fermentation, highlighting their metabolic flexibility under lignin rich conditions. The Indole test was positive in MV11, MV17, and MV22 but negative in MV19. Notably, only MV19 was urease positive, whereas phenylalanine deaminase activity was observed in MV11 and MV22. None of the isolates produced H_2S. Such variability in amino acid metabolism indicates ecological diversification among the isolates.

These results suggest that the isolates possess multiple adaptive traits, including motility, oxidative metabolism, nitrate reduction, and extracellular enzyme production, all of which are advantageous for survival in lignocellulosic environments. Similar biochemical patterns were reported for laccase producing strains by Yadav *et al*. (2014) [42] and Ghosh *et al*. (2021). [12]

3.1.3 Screening of lignin degrading bacteria for ligninolytic enzymes in plates

The potent ligninolytic isolates MV11, MV17, MV19, and MV22 were screened for the production of laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) using agar plates supplemented with chromogenic indicators (Table 6). All isolates exhibited positive reactions, confirming their ligninolytic potential. Specifically, the development of green coloration on ABTS agar and clear halo zones on o-dianisidine agar indicated laccase activity, while halo formation on guaiacol supplemented basal salt agar confirmed MnP activity. Furthermore, decolorization of Azure B dye demonstrated the production of LiP.

Table 6: Screening of lignin degrading bacteria for ligninolytic enzymes in plates

Indicators	MV11	MV17	MV19	MV22
ABTS agar (Laccase)	+ +	+ +	+ +	+ +
o-dianisidine agar (Laccase)	+	+	+ +	+
Basal salt agar containing 0.01% Guaiacol (MnP)	+	+	+ +	+
Azure B agar (LiP)	+	+ +	+ +	+

Notes: "+" weak positive, "+ +" strong positive

For comparative evaluation, the potency index of isolates was assessed on ABTS agar (Table 7 and Photo 1). All isolates produced a characteristic green zone; however, the values varied. The significantly highest potency index was recorded in MV19 (2.32), followed by MV17 (1.90) and MV22 (1.63), while MV11 showed the lowest value (1.36). This indicates that although all isolates are capable of laccase production, MV19 possesses superior ligninolytic activity and was therefore selected for further optimization studies.

Table 7: Screening of lignin degrading bacteria for lignolytic enzymes in plates

Sr. No.	Strain No.	Potency index in ABTS agar
1	MV11	1.36^{d}
2	MV17	1.90^{b}
3	MV19	2.32^{a}
4	MV22	1.63^{c}
S. Em.±		0.017
CD at 5%		0.05
C.V. %		1.9

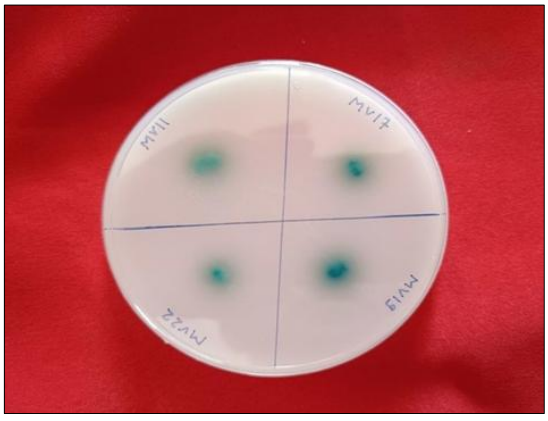

Photo 1: Potency index of isolates on ABTS agar

These findings are consistent with earlier reports where ABTS, o-dianisidine, Azure B, methyl orange, and naphthol were successfully employed as indicators for lignin modifying enzymes (Archibald, 1992; Dhouib *et al.*, 2005; Ravikumar *et al.*, 2012; Rajwar *et al.*, 2016) [3, 5]. In particular, Kaur *et al.* (2018) [15] highlighted 0.08% ABTS as a sensitive and reliable indicator, providing clear visual expression and higher potency index, which supports the present observations.

3.1.4 Identification of potent degrading bacteria

Molecular identification of the most potent ligninolytic bacterial isolate MV19 was carried out using 16S rRNA gene sequencing with a universal primer. The obtained sequence showed 100% homology with *Staphylococcus cohnii* in BLAST analysis. A phylogenetic tree constructed using the NCBI platform (Figure 1) further confirmed its taxonomic placement. The sequence of isolate MV19 was deposited in the NCBI GenBank under the accession number PV876755. Thus, based on morphological, biochemical, and molecular characterization, the ligninolytic bacterial isolate was identified as *Staphylococcus cohnii* NAU MV19.

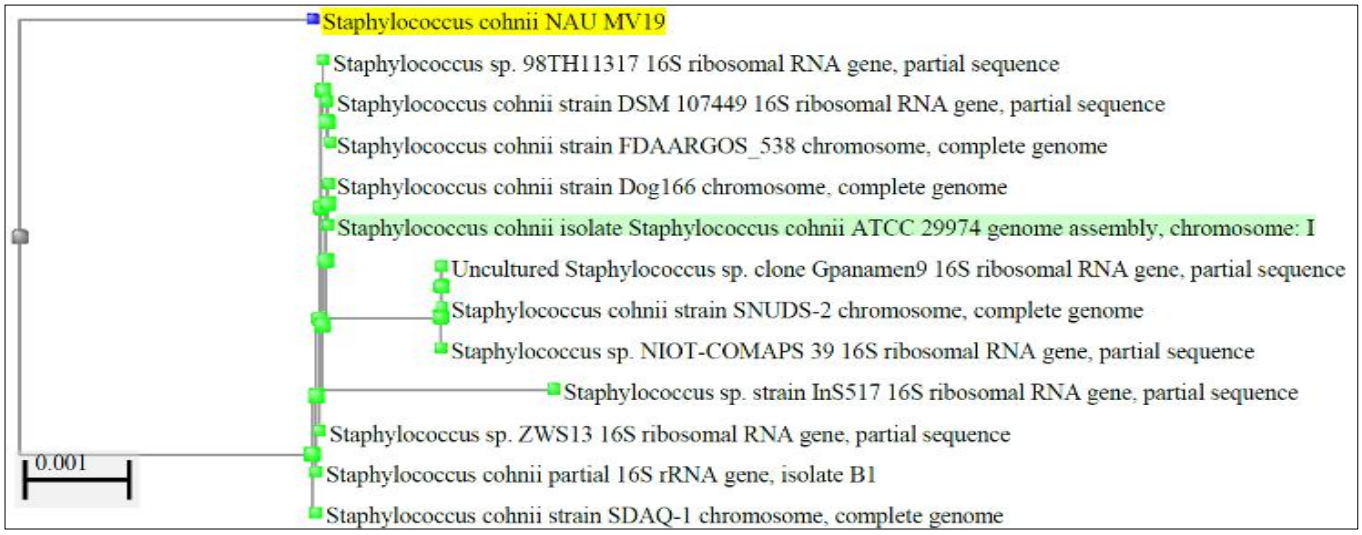

Fig 1: Phylogenetic tree of *Staphylococcus cohnii* NAU MV19 (PV876755)

Similar molecular approaches have been widely employed to identify lignocellulose degrading bacteria. Rawway *et al.* (2018) [30] reported the isolation of ten cellulose degrading strains from Assiut Governorate, Egypt. These Gram-positive, rod-shaped, motile isolates were identified as *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus pumilus*, *Brevibacillus brevis*, and *Brevibacillus parabrevis* using morphological, biochemical, and 16S rRNA gene analysis, with selected sequences deposited in GenBank. Likewise, Batayyib *et al.* (2022) [5] characterized ligninolytic bacteria from Jeddah, Saudi Arabia, and identified *Streptomyces lavendulae* (OL697233.1) and *Priestia aryabhattai* (OL697234.1) as potent lignin peroxidase producers.

The present study identifies *Staphylococcus cohnii* NAU MV19 as a potent ligninolytic bacterium. Its consistent performance across morphological, biochemical, and molecular characterization confirms its stability and highlights its potential for future applications in lignin degradation and biomass valorization.

3.2 Optimization of different physico-chemical parameters for laccase production under submerged conditions

Optimization of carbon, nitrogen sources, pH, and temperature was carried out using the one factor at a time (OFAT) approach to enhance laccase production by *Staphylococcus cohnii* NAU MV19 under submerged fermentation.

3.2.1 Effect of Carbon Sources

Carbon source strongly influenced laccase yield. Among the tested sources, glucose supported maximum activity (19.78 U/ml at 96 h), followed by starch (13.20 U/ml), lactose (11.11 U/ml), and maltose (7.40 U/ml), whereas xylan showed the least activity (7.26 U/ml) (Table 8). Glucose also yielded the highest protein content (3.08 mg/ml) and specific activity (6.42 U/mg) (Figure 2). Activity peaked at 96 h across all carbon sources, after which a decline was observed. These findings confirm glucose as the most effective inducer of laccase, consistent with earlier reports where glucose enhanced laccase production in *Bacillus subtilis* (Abd Zaid and Hashim, 2016 and Poornima and Velan, 2020) [1, 27].

Table 8: Effect of different carbon sources on laccase enzyme production at different incubation periods

Sr. No.	Carbon sources	Laccase enzyme activity (U/ml)				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
1	Lactose	7.67^{c}	9.68^{c}	9.98^{c}	11.11^{c}	6.19^{c}
2	Starch	12.18^{b}	12.46^{b}	12.82^{b}	13.20^{b}	9.35^{b}
3	Xylan	6.22^{d}	5.25^{f}	6.34^{e}	7.26^{d}	5.32^{e}
4	Maltose	6.07^{d}	6.63^{d}	6.84^{d}	7.40^{d}	5.48^{d}
5	Glucose	16.93^{a}	16.83^{a}	17.29^{a}	19.78^{a}	14.40^{a}
S. Em ±		0.06	0.04	0.03	0.05	0.02
CD @ 5%		0.20	0.14	0.10	0.17	0.08
CV %		1.29	0.86	0.59	1.04	0.65

Note: Different letters in the same column indicate significant differences.

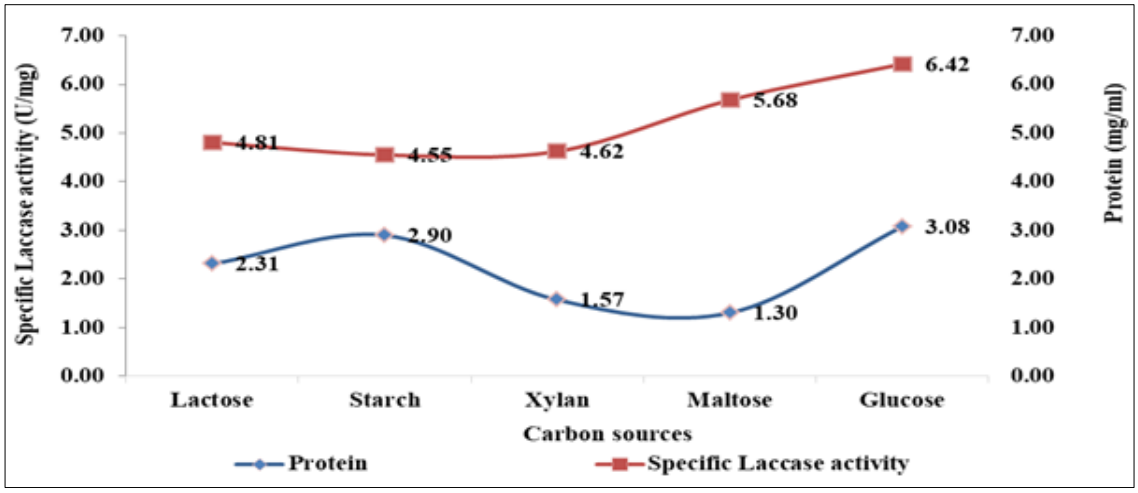

Fig 2: Specific Laccase activity (U/mg of protein) and protein (mg/ml) with different carbon sources

3.2.2 Effect of Nitrogen Sources

Nitrogen source is a critical factor influencing laccase enzyme production in lignin degrading bacteria. A significantly higher laccase activity of 14.04, 14.35, 16.12, 18.19 and 16.97 U/ml were observed in urea at 24, 48, 72, 96 and 120 hrs after incubation, respectively (Table 9). Among different nitrogen sources ammonium tartarate showed lowest laccase enzyme activity at all incubation periods. Among different nitrogen sources, urea was found suitable nitrogen source for high enzyme activity (18.19 U/ml) followed by peptone (7.47 U/ml). These finding suggest that urea, an organic nitrogen source is the most suitable for maximizing laccase production under submerged conditions. The superior performance of urea may be attributed to its bioavailable nitrogen, which supports rapid microbial growth and enzyme synthesis. In contrast, inorganic nitrogen like ammonium tartarate may suppress enzyme production due to catabolic repression. The corresponding protein content (3.65 mg/ml) and specific activity (4.98 U/mg) were also maximal with urea (Figure 3). Similar trends were reported by Tamilvanan (2020) [35] and Ding *et al.* (2012) [11], who observed enhanced laccase yields with organic nitrogen sources, underscoring the importance of readily assimilable nitrogen in promoting enzyme synthesis.

Table 9: Effect of different nitrogen sources on laccase enzyme production at different incubation periods

Sr. No.	Nitrogen sources	Laccase enzyme activity (U/ml)				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
1	Peptone	3.86^{b}	4.17^{b}	4.60^{b}	7.47^{b}	6.33^{b}
2	Gelatin	2.09^{c}	2.85^{c}	3.46^{c}	4.61^{c}	3.90^{c}
3	Urea	14.04^{a}	14.35^{a}	16.12^{a}	18.19^{a}	16.97^{a}
4	Ammonium tartarate	1.30^{d}	1.49^{d}	2.11^{d}	2.98^{d}	1.98^{d}
S. Em ±		0.02	0.01	0.03	0.01	0.05
CD @ 5%		0.06	0.04	0.09	0.05	0.19
CV %		0.65	0.46	0.74	0.34	1.4

Note: Different letters in the same column indicate significant differences

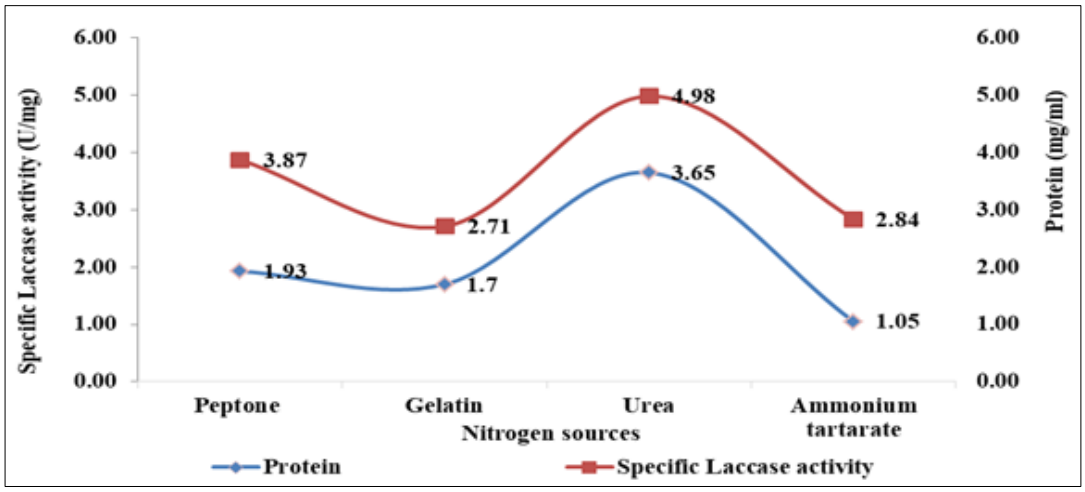

Fig 3: Specific Laccase activity (U/mg of protein) and protein (mg/ml) with different nitrogen sources

3.2.3 Effect of pH

A production of laccase is strongly influenced by environmental pH, which affects both the synthesis and catalytic efficiency of the enzyme. pH alters the ionization of amino acid residues at the enzyme's active site, thereby impacting substrate binding and electron transfer during enzymatic reactions.

The data presented in Table 10 revealed that a significantly higher laccase activity of 13.70 U/ml was recorded at pH 6 followed by pH 7 (6.12 U/ml), while lowest laccase activity was found in pH 9 (4.23) at 96 hrs after incubation. Laccase activity in all pH treatments increased gradually up to 96 hrs, after which a decline was noted, indicating that this time point represented the peak production. Moreover, specific

laccase activity of (3.98 U/mg) and protein (3.44 mg/ml) was found at pH 6, while lowest specific laccase activity was found at pH 9 (2.38 mg/ml) and protein content (1.78 mg/ml) (Figure 4) indicating a negative effect of alkaline pH on enzyme synthesis. Our results are aligns with previous findings in *Bacillus cereus* and *Bacillus licheniformis* (Lu *et al*., 2013 and Oyedeji *et al*., 2025) [19, 22], reinforcing the preference of bacterial laccases for slightly acidic environments.

Table 10: Effect of different pH on laccase enzyme production at different incubation periods

Sr. No.	pH	Laccase enzyme activity (U/ml)				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
1	5	2.40^{c}	3.69^{c}	4.50^{c}	5.67^{c}	4.40^{c}
2	6	10.73^{a}	11.86^{a}	12.85^{a}	13.70^{a}	12.50^{a}
3	7	3.81^{b}	4.59^{b}	4.99^{b}	6.12^{b}	5.15^{b}
4	8	2.12^{d}	3.12^{d}	3.66^{d}	4.58^{d}	4.11^{d}
5	9	1.68^{e}	2.84^{e}	3.21^{e}	4.23^{e}	3.13^{e}
S. Em ±		0.02	0.08	0.05	0.03	0.09
CD @ 5%		0.07	0.27	0.15	0.08	0.28
CV %		0.94	2.88	1.47	0.71	2.66

Note: Different letters in the same column indicate significant differences

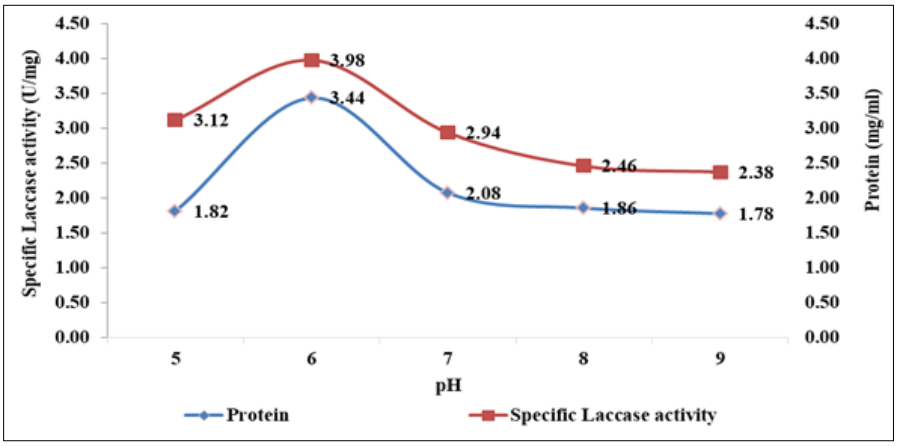

Fig 4: Specific Laccase activity (U/mg of protein) and protein (mg/ml) with various pH

3.2.4 Effect of Temperature

Temperature is a key environmental factor that significantly influences enzyme activity and microbial metabolism.

The results showed that *Staphylococcus cohnii* was capable of producing laccase across all tested temperature conditions (Table 11). However, a significant variation in enzyme activity was observed depending on the incubation temperature. A significantly higher laccase activity was recorded at 30±2 °C (25.67 U/ml) followed by 40±2 °C (18.08 U/ml), 20±2 °C (14.58 U/ml) whereas, the lowest laccase activity was recorded at 50±2 °C (13.45 U/ml) at 96 hrs after incubation. The highest specific activity (8.94 U/mg) and protein yield (2.87 mg/ml) (Figure 5) were also recorded at 30±2 °C. These results agree with earlier reports on *Bacillus subtilis* and *Enterobacter cloacae* where mesophilic conditions favored maximum laccase secretion (Muthukumarasamy *et al.*, 2015 and Hemaraju *et al.*, 2021) [20, 13].

Table 11: Effect of different temperature on laccase enzyme production at different incubation periods

Sr. No.	Temperature	Laccase enzyme activity (U/ml)				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
1	20±2° C	9.93^{b}	10.84^{b}	14.21^{b}	14.58^{b}	11.51^{b}
2	30±2° C	12.84^{a}	16.63^{a}	21.07^{a}	25.67^{a}	14.55^{a}
3	40±2° C	8.56^{c}	12.65^{c}	15.15^{c}	18.08^{c}	11.29^{c}
4	50±2° C	5.59^{d}	10.07^{d}	12.16^{d}	13.45^{d}	9.64^{d}
S. Em ±		0.06	0.10	0.07	0.04	0.08
CD @ 5%		0.13	0.33	0.23	0.23	0.24
CV %		1.12	1.43	0.65	0.72	0.92

Note: Different letters in the same column indicate significant differences

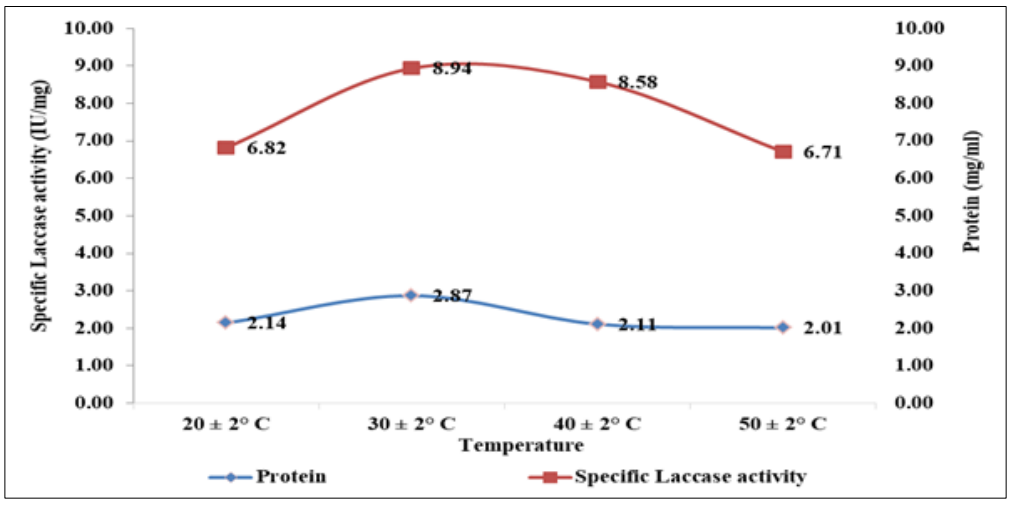

Fig 5: Specific Laccase activity (U/mg) and protein (mg/ml) with different temperature

3.2.5 Integrated Optimization

Under optimized conditions (glucose, urea, pH 6.0, 30±2 °C), maximum laccase activity (26.74 U/ml) (Figure 6) was achieved at 96 h, along with a specific activity of 10.01 U/mg and protein content of 2.67 mg/ml (Figure 7). The temporal pattern showed enzyme synthesis peaked during the late exponential to early stationary phase, consistent with the secondary metabolite nature of laccase.

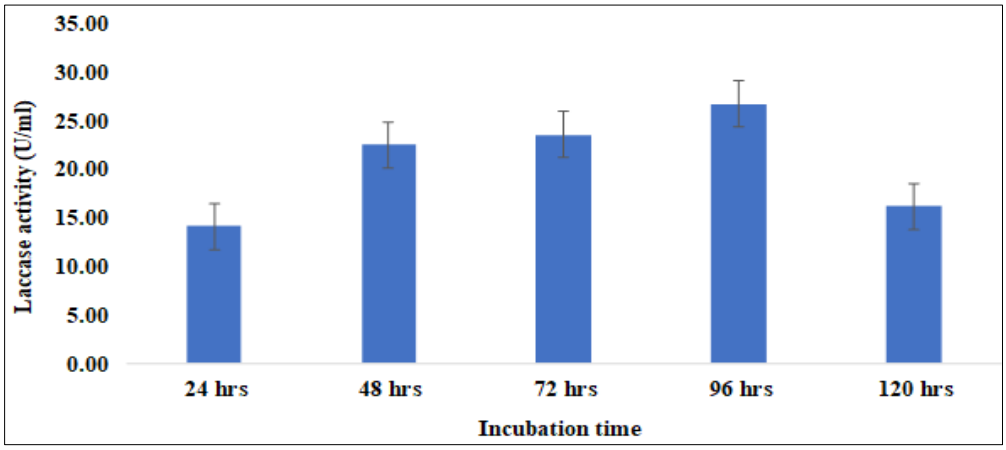

Fig 6: Laccase activity with optimized conditions

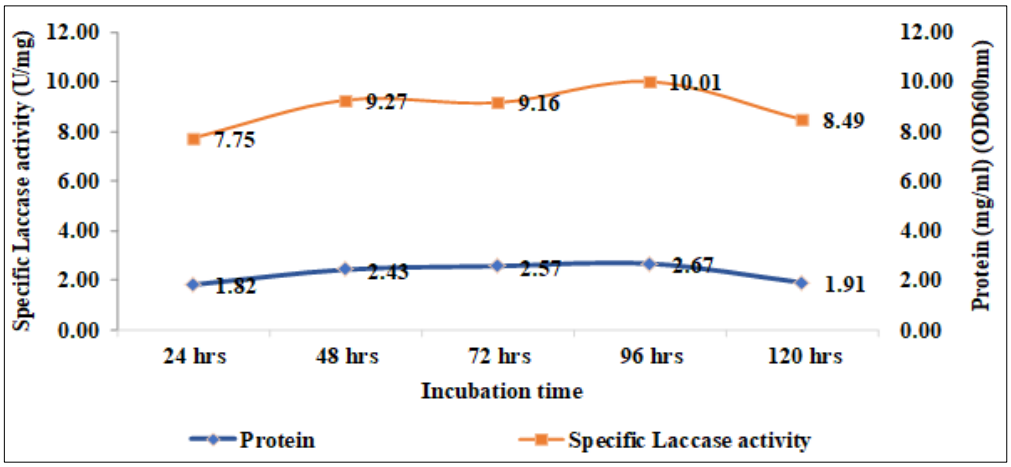

Fig 7: Specific Laccase activity (U/mg) and protein (mg/ml) with optimized conditions

4. Conclusion

The present study isolated 25 bacteria from the Dangs region of Gujarat, out of which four showed strong ligninolytic activity. Among them, *Staphylococcus cohnii* NAU MV19 was identified as the most potent strain. Optimization revealed glucose, urea, pH 6.0, and 30±2 °C as the best conditions, yielding maximum laccase activity (26.74 U/ml). These results demonstrate the potential of MV19 for lignin degradation and related biotechnological applications.

References

1. Abd Zaid AM, Hashim AJ. Optimal conditions for spore-bound laccase production from local isolate of *Bacillus subtilis*. Al-Qadisiyah J Pure Sci. 2016;21(3):42-54.
2. Adarsh K, Chandra R. Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. Heliyon. 2020;6:170-178.
3. Archibald FS. A new assay for lignin-type peroxidases employing the dye azure B. Appl Environ Microbiol. 1992;58(9):3110-3116.
4. Ball AS, Betts WB, McCarthy AG. Degradation of lignin-related compounds by Actinomycetes. Appl Environ Microbiol. 1989;55:1642-1646.
5. Batayyib RS, Al-Twaty NH, El-Hamshary OI. Isolation and molecular identification of lignin peroxidase-producing bacterial isolates from Jeddah City. Egypt Pharm J. 2022;21(3):338-346.
6. Bohacz J, Kowalska KT. Modification of post-industrial lignin by fungal strains of the genus *Trichoderma* isolated from different composting stages. J Environ Manag. 2020;266:3-11.
7. Bugg TD, Ahmad M, Hardiman EM, Rahmanpour R. Pathways for degradation of lignin in bacteria and fungi. Nat Prod Rep. 2011;28(12):1883-1896.
8. Campbell MM, Sederoff RR. Variation in lignin content and composition: mechanisms of control and implications for the genetic improvement of plants. Plant Physiol. 1996;110(1):3-13.
9. Chandra R, Yadav S, Kumar V. Microbial degradation of lignocellulosic waste and its metabolic products. Environ Waste Manag. 2015;249-298.
10. Dhouib A, Hamza M, Zouari H, Mechichi T, Hmidi R, Labat M, Martinez M, Sayadi S. Screening for ligninolytic enzyme production by diverse fungi from Tunisia. World J Microbiol Biotechnol. 2005;21:1415-1423.
11. Ding Z, Peng L, Chen Y, Zhang L, Shi G. Production and characterization of thermostable laccase from the

mushroom *Ganoderma lucidum* using submerged fermentation. Afr J Microbiol Res. 2012;6(6):1147-1157.
12. Ghosh D, Khan S, Sharadamma N. Isolation and characterization of lignin-degrading bacteria. J Adv Sci Res. 2021;12(4):140-149.
13. Hemaraju S, Narasegowda PN, Bharathi TR. Optimization of process parameters of laccase production by *Enterobacter cloacae* strain T137 in submerged fermentation. Res J Biotechnol. 2021;16(1):26-52.
14. Huang XF, Santhanam N, Badri DV, Hunter WJ, Manter DK, Decker SR, Vivanco JR, Reardon KF. Isolation and characterization of lignin-degrading bacteria from rainforest soils. Biotechnol Bioeng. 2013;110(6):1616-1626.
15. Kaur H, Kapoor S, Sharma S. An efficient method for qualitative screening of ligninolytic enzyme potential of *Ganoderma lucidum*. Int J Curr Microbiol Appl Sci. 2018;7(8):2442-2459.
16. Keerthana S, Kalaiselvi P, Maheshwari M, Kalaiselvi T. Isolation and screening of lignin and cellulose-degrading proficient microbial strains from diverse biotic substrates based on qualitative traits. Int J Curr Microbiol Appl Sci. 2019;8(7):475-483.
17. Li J, Wang P, Salam N, Li X, Ahmad M, Tian Y, Duan L, Huang L, Xiao M, Mou X, Li W. Unraveling bacteria-mediated degradation of lignin-derived aromatic compounds in a freshwater environment. Sci Total Environ. 2020;749:1-40.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-275.
19. Lu L, Wang TN, Xu TF, Wang JY, Wang CL, Zhao M. Cloning and expression of thermo-alkali-stable laccase of *Bacillus licheniformis* in *Pichia pastoris* and its characterization. Bioresour Technol. 2013;134:81-86.
20. Muthukumarasamy N, Jackson B, Raj JA, Sevanan M. Production of extracellular laccase from *Bacillus subtilis* MTCC 2414 using agroresidues as a potential substrate. Biochem Res Int. 2015;20:1-9.
21. Niku-Paavola ML, Raaska L, Itavaara M. Detection of white-rot fungi by a nontoxic stain. Mycol Res. 1990;94:27-31.
22. Oyedeji BA, Oloye NR, Adebajo SO, Taiwo MO, Folarin BT, Akintokun AK. Optimization and production of laccase enzyme by *Bacillus cereus* isolated from effluent of paint producing industry. Bull Natl Res Cent. 2025;49(1):27.
23. Palmqvist E, Hagerdal B. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresour Technol. 2000;74:25-33.
24. Perez J, Munoz-Dorado J, De la Rubia TDLR, Martinez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int Microbiol. 2002;5:53-63.
25. Petti CA. Detection and identification of microorganisms by gene amplification and sequencing. Clin Infect Dis. 2007;44:1108-1114.
26. Pollegioni L, Tonin F, Rosini E. Lignin-degrading enzymes. FEBS J. 2015;282(7):1190-1213.
27. Poornima P, Velan M. A novel laccase-producing *Brevundimonas* sp. MVSP from paper and pulp industry wastewater. J Environ Biol. 2020;39(4):447-453.
28. Rajwar D, Joshi S, Rai JPN. Ligninolytic enzymes production and decolorization potential of native fungi isolated from pulp and paper mill sludge. Nat Environ Pollut Technol. 2016;15(4):1241-1248.
29. Ravikumar G, Gomathi D, Kalaiselvi M, Uma C. Production, purification and partial characterization of laccase from the mushroom *Hypsizygus ulmarius*. Int J Pharm Bio Sci. 2012;3:355-365.
30. Rawway M, Ali SG, Badawy AS. Isolation and identification of cellulose-degrading bacteria from different sources at Assiut Governorate (Upper Egypt). J Ecol Health Environ. 2018;6(1):15-24.
31. Rogalski JERZY, Lundell T, Leonowicz ANDRZEJ, Hatakka A. Production of laccase, lignin peroxidase and manganese-dependent peroxidase by various strains of *Trametes versicolor* depending on culture conditions. Acta Microbiol Pol. 1991;40(3-4):221-234.
32. Sasikumar V, Priya V, Shankar CS, Sekar SD. Isolation and preliminary screening of lignin-degrading microbes. J Acad Ind Res. 2014;3(6):291-294.
33. Siva J, Kishore K, Hema E. Screening, isolation and biochemical characterization of laccase-producing bacteria from contaminated sites. Int J Pharm Sci Rev Res. 2021;71(2):117-121.
34. Sumranwanich T, Amosu E, Chankhamhaengdecha S, Phetruen T, Loktumraks W, Ounjai P, Harnvoravongchai P. Evaluating lignin degradation under limited oxygen conditions by bacterial isolates from forest soil. Sci Rep. 2024;14(1):1-14.
35. Tamilvanan N. Optimization, production, purification of laccase enzyme from *Bacillus* sp. J New Dev Chem. 2020;2(4):35-43.
36. Tandel A. Isolation, characterization and identification of cellulose-decomposing bacteria [Master's thesis]. Sardar Patel Univ, Vidhyanagar; 2013.
37. Teck NA, Gek CN, Adeline SMC. A quantitative method for fungal ligninolytic enzyme screening studies. Asia-Pac J Chem Eng. 2011;6:589-595.
38. Tian JH, Pourcher AM, Peu P. Isolation of bacterial strains able to metabolize lignin and lignin-related compounds. Lett Appl Microbiol. 2016;63(1):30-37.
39. Utarti E, Triono ISM, Rohma A, Labes A, Arimurti S. Isolation of lignocellulolytic bacteria from Erek-Erek Geoforest Ijen Geopark, Indonesia. BIO Web Conf. 2024;101:1-13.
40. Wolfenden BS, Wilson RI. Radical cations as reference chromogens in kinetic studies of one-electron transfer reactions. J Chem Soc Perkin Trans 2. 1982;11:805-812.
41. Yadav M, Yadav HS. Applications of ligninolytic enzymes to pollutants, wastewater, dyes, soil, coal, paper and polymers. Environ Chem Lett. 2015;13:309-318.
42. Yadav S, Gangwar N, Mittal P, Sharama S, Bhatnagar T. Isolation, screening and biochemical characterization of laccase-producing bacteria for degradation of lignin. Int J Educ Sci Res. 2014;1(3):17-21.