

# Isolation, Screening and Characterization of a Novel Extracellular Laccase Producing *Bacillus* sp.

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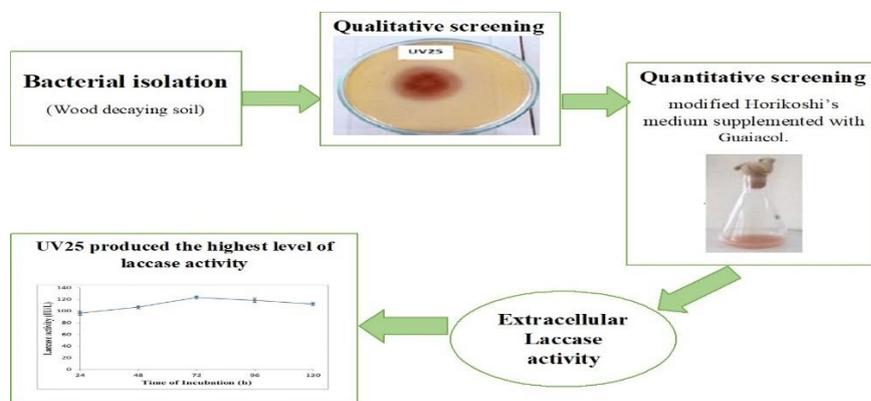
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## Abstract

Laccase is a unique, green biological catalyst with several applications due to its high prolific activity. They have sparked widespread attention as potential industrial enzymes in a range of industries, including bioremediation in pulp and paper, textiles, and food. Laccase has also shown enormous potential for anti-cancer treatment due to its proliferation inhibitory actions, which are still being explored. Isolation and identification of laccase producing bacteria, becomes an essential one. The laccase producing bacterial colonies were isolated from the collected samples by serial dilution technique. Seven samples comprising of decaying wood soil, ant- induced soil and soil contaminated with dyes and effluents of dyeing industry were collected from Kurukshetra and Panipat districts of Haryana. Preliminary screening of bacterial strains was carried out by spreading of these samples on nutrient agar medium supplemented with 0.1% guaiacol followed by incubation at 37°C for 72 h resulted in 56 laccase positive colonies. The formation of a reddish brown color demonstrates that the bacterial strains are capable of producing laccase. These isolated bacterial colonies were subjected to quantitative screening for extracellular laccase production in submerged fermentation using modified Horikoshi's medium supplemented with Guaiacol. The bacterial isolates exhibited differences in laccase production and the isolate UV25 showed the highest level of laccase activity 250.04 IU/L among all the isolates. This bacterial isolate was examined for morphological and biochemical characteristics according to Bergey's Manual of Systematic Bacteriology and it was identified as *Bacillus* species.

**Keywords:** Extracellular Laccase, *Bacillus* sp., Soil sample, Screening, Guaiacol

## Graphical Abstract



## 1. INTRODUCTION

Laccase (Lac, EC1.10.3.2) is a member of the polyphenol oxidases referred to as the blue multi-copper oxidases that exhibit the inherent properties of oxidizing a range of phenolic substrates (Elsaba et al., 2023; Ryan et al., 2003). They include four distinct copper atoms with differing electro-paramagnetic resonance (EPR) and spectroscopic characteristics (Mathur et al., 2021). Their specificity towards substrate is broader compared to other enzymes; hence, their ability to hydrolyze a wide range of inorganic and organic compounds, aromatic and non-aromatic compounds such as phenolic and non-phenolic groups, hydroxyl and aromatic amine etc. (Ansari et al., 2021, Oyedeji et al., 2025). Owing to their broad substrate specificity, laccases have gained much attention as potential industrial enzymes in various industries

involved in bioremediation, biodegradation, decolorization of environmental polluted dyes and pharmaceutical sector also. Very recently, laccases have found a potential use in the field of therapeutics, particularly against cancer.

Dye wastewater has emerged as one of the most harmful industrial sewage due to huge number of dyes and intermediates, particularly increases the cancer risk. Wastewater from the printing and dyeing industry is discharged into freshwater without treatment, threatening the growth of aquatic organisms and microorganisms (Mishra and Maiti, 2018), and destroying the self-purification of water bodies (Tkaczyk et al., 2020; Gowri et al., 2014). Simultaneously, azo and anthraquinone dyes create a range of carcinogenic aromatic amines during specialized breakdown, which can lead to cancer, mutagenesis, and reproductive damage (Ali et al., 2019). For the treatment of dyestuffs, biodegradation offers several benefits over conventional physical and chemical processes, including cost effectiveness, high efficiency, and environmental protection. Laccase is the best candidate for dye degradation because of its diverse range of substrates and pollution-free products.

Laccase is commonly found in various fungi and higher plants (Olmeda et al., 2021). It is mainly produced from fungi, especially white rot, and has been extensively exploited for the application in industrial processes due to their high redox potential. The commercial production of fungal laccases has many problems, such as a long culture period and high cost. On contrary, bacterial laccases have numerous distinctive properties over fungal laccases, including stability at high temperatures, pH and high salt concentration operating conditions in industries which making them of a major interest (Du et al., 2015; Wang et al., 2011). In addition, they have some additional advantages such as enzyme production in a short time, thermostability and easy to clone and express in the host with suitable manipulation that promote their cost-effective use in industrial applications (Fernandes et al., 2014). Moreover, in term of bioremediation, bacterial laccases displayed leading role in decomposing lignin. Therefore, researchers turned their attention to bacterial laccase.

The screening, isolation and biochemical characterization of Laccase producing bacteria from soil was carried out to assess the diversity of Lignocellulose degrading bacteria/ for evaluation of degradation of xenobiotics to solve environmental issues that making them as essential one. The objective of this study is to isolate, screen and produce bacterial laccase of ligninolytic activity. Total of 56 bacterial strains were isolated from soil samples collected from different regions of Kurukshetra and panipat district on 0.1% guaiacol containing nutrient agar.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and reagents

Guaiacol and other reagents were procured from Hi Media Laboratories, Mumbai, India and were of analytical grade.

### 2.2. Sample collection

Seven samples were aseptically collected in sterilized containers from the decaying wood soil, ant- induced soil and soil contaminated with textile dyes and effluents, from the Kurukshetra and Panipat districts of Haryana, India. Collected samples were brought immediately to the laboratory in ice pack and were stored in a refrigerator for the isolation of laccase-producing bacterial strains.

### 2.3. Isolation of laccase producing microorganisms

For Isolation, collected samples were subjected to isolation by serial dilution technique using standard microbiological procedures (Lin and Stephenson, 1998). Prior to the isolation process, samples were placed at room temperature for normalization of their temperature. Dried soil samples were filtered and 1.0 g was weighed out for serial dilution protocol. About 100  $\mu$ l of  $10^{-3}$ ,  $10^{-5}$  and  $10^{-6}$  diluted samples were spread on petri plates containing nutrient agar (composed of peptone 5.0 g/L, beef extract 3.0 g/L and agar 25.0 g/L, pH 7.0) supplemented with 0.1% (v/v) guaiacol (Degryse et al., 1978) with the help of a sterile spreader. The plates were incubated at 37 °C for 72 h. The colonies showing reddish brown colored zone (halo) formed by the oxidation of guaiacol on nutrient agar plates were evaluated as laccase producers. The isolated colonies thus obtained were maintained on nutrient agar plates at 4°C for further analysis. Fifty-six strains were isolated and were designated as UV1 to UV56.

#### 2.4. Qualitative screening of yeast for laccase production

All the isolated bacterial strains were subjected to qualitative assay by streaked again on the same medium (Degryse et al., 1978) to confirm the laccase producing isolates. The laccase positive colonies were further sub-cultured on same guaiacol amended nutrient agar plates to obtain pure cultures. Again, these purified colonies were maintained on nutrient agar plates at 4°C for subsequent use as inoculums and subcultured after every 5 days. The most potent bacterial isolate was then subjected to cell morphology and biochemical tests.

#### 2.5 Quantitative screening of the laccase positive bacterial strains for laccase production

The isolated laccase producing bacterial strains were also quantitatively screened by measuring their laccase activity. The inoculum of each isolated strain was prepared by culturing it in the nutrient broth containing 0.1% guaiacol (v/v) at 37 °C under shaking at 200 rpm for 18 h in an orbital shaker. The laccase production was carried out in triplicates in modified Horikoshi medium (peptone 5.0 g/L, beef extract 5.0 g/L, yeast extract 5.0 g/L, KNO<sub>3</sub> 5.0 g/ KH<sub>2</sub>PO<sub>4</sub> 0.2 g/L, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.1 g/L, pH 7.0) containing 1.0 ml/L guaiacol. The production medium was inoculated with 2% of 18 h old inoculum and incubated in an orbital shaker at 200 rpm at 37 °C for 72 h respectively. After 72 h of incubation, culture filtrates were centrifuged at 10,000 x g for 10 min at 4 °C to collect the clear supernatant which was used as the crude extracellular laccase enzyme preparation.

#### 2.6 Laccase activity assay

Laccase activity was determined by following the method of Das et al. (1997). The reaction mixture for laccase assay containing 1.9 ml of 0.1 M sodium acetate buffer (pH 4.0), 0.5 ml of 0.1 M guaiacol and 0.1 ml enzyme. The reaction mixture was incubated in a water bath at 37 °C for 15 min. The enzyme and substrate controls were run simultaneously. The enzyme control contained buffer and enzyme but lacked guaiacol. The substrate control contained buffer and guaiacol but was devoid of enzyme. The absorbance of the resulting-colored product in test, enzyme control and substrate control tubes were read against distilled water at 470 nm in a Spectrophotometer. The absorbance of enzyme and substrate controls was subtracted from the absorbance of test. Each enzyme assay was performed in duplicates. The enzyme activity was calculated using an extinction coefficient of 6740 M<sup>-1</sup>cm<sup>-1</sup> and expressed in IU/L. One enzyme unit was defined as the amount of enzyme catalyzing the production of one μmol of colored product per min under the specified enzyme assay conditions.

The laccase activity in UmL<sup>-1</sup> was calculated using this formula:  $E.A = A \times V / t \times e \times v$

Where, E.A = Enzyme Activity, A = Absorbance, V = Total mixture volume (mL), v = enzyme volume (mL), t = incubation time (mins), e = extinction coefficient for guaiacol (0.6740 μM/ cm)

#### 2.7. Time course study of laccase production from the selected strain

To investigate the optimum incubation time for laccase production, quantitative assay was performed for selected bacterial isolate by inoculating 2% of 18 h old inoculum of isolate in modified Horikoshi medium in an orbital shaker at 200 rpm at 37 °C. The samples were withdrawn at time intervals of 24 h and were studied for laccase activity as per standard protocol.

#### 2.8. characterization of the selected bacterial isolate

The selected bacterial isolate UV25 was identified on the basis of morphological, physiological, and biochemical analysis. The morphological identification involved the microscopic analysis of bacterial colonies (color, shape, and appearance) and cell morphological features (shape and motility) and Gram staining of the isolated strain were performed according to standard protocol. The physiological tests involved the effect of temperature, pH and salt concentration on the growth of the bacterium. The biochemical tests were performed for the production of lipase, gelatin liquefaction, starch hydrolysis, catalase, urease, citrate utilization, and indole, triple sugar iron agar test and carbohydrate fermentation tests (using HiCarbo™ kit purchased from HiMedia Laboratory, India). These were carried out on 24 h old bacterial cell culture according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). The carbohydrate fermentation tests are based on the principle of pH change and substrate utilization. An aliquot (50 μl) of 24 h old bacterial inoculum (0.5 O.D. at 620 nm) was inoculated in each well and incubated at 37 °C for 24 h. On incubation, bacteria undergo metabolic changes which are indicated by a color change in the media that are interpreted visually. The morphological, physiological and biochemical tests helped in identification of the bacterial isolate at the genus level.

### 3. RESULTS AND DISCUSSION

#### 3.1. Isolation and Screening of Laccase producing bacteria

Qualitative screening showed that total of 56 bacterial colonies was isolated for laccase production which were named as UV1 to UV56. All the isolates were primarily screened for laccase production on nutrient agar medium containing 0.1% guaiacol using plate assay method. All the plates were incubated at 37°C for 48 hrs. This was confirmed by a reddish colouration produced during the oxidation of the substrate (0.1 % guaiacol). The laccase positive isolates were further confirmed by streaking the colonies on the same medium and looking for the appearance of brown colored colonies (Fig. 1). Similar result was reported by Devasia and Nair (2016), Rajput and Mishra (2019) and Sharma et al. (2020). This finding may be due to the isolates ability to metabolize complex organic and inorganic matter released in their environments using their laccase enzyme, indicating the laccase generating nature of the isolate.

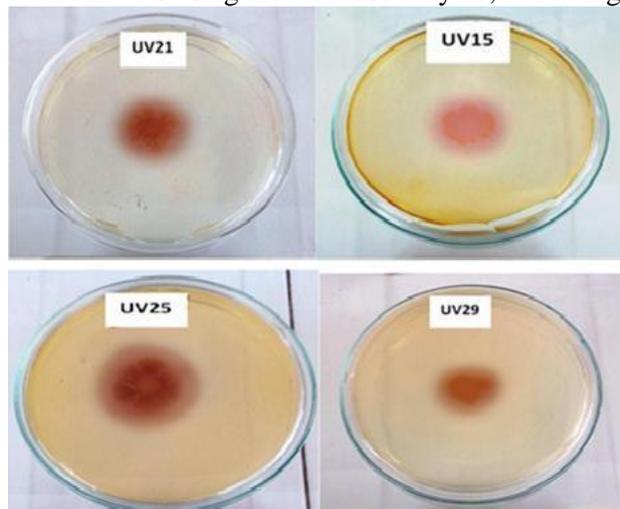


Fig. 1 four selected laccase positive isolates showing reddish brown colored colonies on nutrient agar-guaiacol plates

All the positive isolates were evaluated quantitatively for their potential to produce extracellular laccase by using screening media on Horikoshi medium containing 0.1% guaiacol in SmF for a period of 72 h with agitation at 200 rpm. The results of quantitative screening revealed marked differences among laccase positive bacterial isolates with regard to laccase secretion into the culture medium. The extracellular laccase activity of all the isolates ranged from 1.84 to 240.04 IU/L (Table 1). Among all these, four isolates viz. UV15, UV21, UV25 and UV29 showed prominent extracellular laccase activity in higher levels (174.52, 232.00, 240.04 and 213.64 IU/L, respectively) as compared with other isolates after 72 h of incubation. Since the bacterial isolate UV25 produced the highest amount of laccase among all the isolates in quantitative enzyme assay, it was selected as the potential strain for identification and further characterization. It was isolated from the soil impregnated with decaying wood.

Table 1. Quantitative screening of laccase positive isolates for extracellular laccase production

Isolate No.	Laccase activity (IU/L)	Isolate No.	Laccase activity (IU/L)	Isolate No.	Laccase activity (IU/L)
UV1	34.48	UV20	45.64	UV39	17.44
UV2	28.56	<b>UV21</b>	<b>232.00</b>	UV40	28.00
UV3	21.32	UV22	16.88	UV41	21.68
UV4	18.92	UV23	5.56	UV42	140.96
UV5	20.20	UV24	8.16	UV43	32.08
UV6	27.80	<b>UV25</b>	<b>240.04</b>	UV44	14.64
UV7	17.44	UV26	17.60	UV45	14.08
UV8	10.36	UV27	16.12	UV46	6.12
UV9	21.88	UV28	18.72	UV47	5.36
UV10	100.32	<b>UV29</b>	<b>213.64</b>	UV48	114.04
UV11	2.76	UV30	17.80	UV49	7.04
UV12	8.16	UV31	29.28	UV50	1.84
UV13	160.04	UV32	12.44	UV51	12.96
UV14	27.80	UV33	16.68	UV52	21.32

<b>UV15</b>	<b>174.52</b>	UV34	17.60	UV53	14.64
UV16	10.36	UV35	2.76	UV54	20.20
UV17	30.04	UV36	15.56	UV55	78.64
UV18	13.36	UV37	15.56	UV56	21.52
UV19	42.48	UV38	15.76		

The laccase activity displayed by UV25 (240.04 IU/L) is higher than earlier reported Desai (2017) and Verma et al. (2015)'s yield of 0.694 IU/L and 58 IU/L produced by Indigenous bacterial strain SB1 and *Pseudomonas putida* LUA15.1, but lower than that of Mehandia et al. (2020) who reported a yield of 110 IU/ml produced by *Alcaligenes faecalis* XF1. Differences in yield might be due to varied media composition alongwith modifications in the conditions during production. However, the assay has proved the effectiveness of UV25 isolate to oxidize guaiacol rapidly and secrete more functional laccase enzymes that are closely linked to its oxidation.

### 3.2. Time course study of laccase Production by selected strain

Laccase production by UV25 isolate was done using the best conditions in which laccase was optimally produced. The results showed that laccase production increased significantly with time until day 3, when it reached its peak. Maximum laccase activity was recorded as  $123.34 \pm 2.72$  IU/L at a period of 72 h, followed by a decline on further incubation (Fig. 2). enzyme production and yield highly depend on cultivation conditions medium composition, fermenting organism, and other parameters. However, a decrease in enzyme production was noticed immediately after day 72 h in this study. This decrease may be due to the death of viable cells and the accumulation of by-products in the production medium (Adelabu et al., 2024). Previously, the optimum laccase production from bacteria under SmF was documented after incubation for a period of 24 h (Telke et al., 2011; Verma et al., 2015), 42 h (Rajeswari and Bhuvaneshwari, 2016), 48 h (Rajeswari et al., 2015), 96 h (Kumar et al., 2020; Mehandia et al., 2020; Sondhi et al., 2015), and 12 days (Edoamodu and Nwodo, 2022).

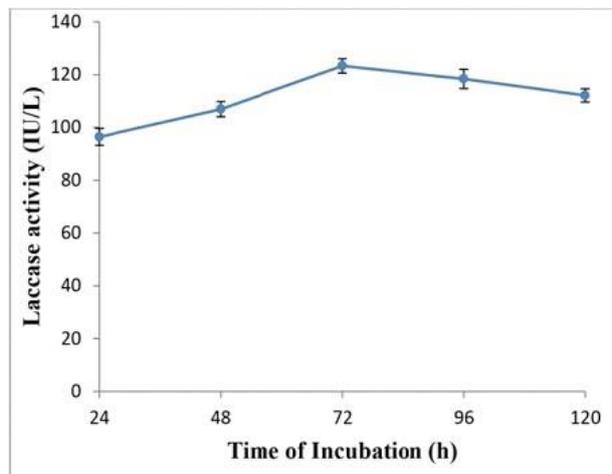


Fig. 2 Time course analysis of selected UV25 strain

### 3.3 The biochemical characterization of UV25 laccase-producing isolate

Most potent laccase-producing bacterial isolate UV25 was selected for biochemical characterization. The identification of bacterial isolate UV25 was performed by analyzing the morphological features of its colony and cells, physiological parameters (effect of temperature, pH and salt concentration on the bacterial growth), biochemical assessments, and molecular investigation of 16S ribosomal RNA gene. The results of these experiments have been documented in the table 2.

On visual examination, the colonies formed by the UV25 bacterial isolate were found to exhibit smooth appearance, circular shape, large size, and white color. On microscopic examination, the bacterial cells were found to be rod shaped, motile, and spore forming. The analysis of the bacterial strain by Gram staining showed it Gram positive (Fig. 3). The physiological characteristics revealed that bacterial cells were able to grow in a medium of pH 5.0-8.0 and temperature 25-45°C under aerobic conditions. The optimum growth occurred at pH 7.0 and a temperature of 37°C. The bacterial cells could tolerate NaCl

concentrations ranging from 1.0 to 5.0% indicating the salt tolerant nature of the strain. The qualitative biochemical assessment of the isolate demonstrated its ability to produce lipase, urease, catalase, and indole. It could liquefy gelatin, hydrolyze starch and utilize citrate. The triple sugar iron agar test was also positive for this strain. The evaluation of the ability of the isolate UV25 to ferment various carbohydrates using HiCarbo™ kit (procured from HiMedia Laboratories Pvt. Ltd.) revealed that it could ferment all the carbohydrates, except ONPG and malonate (Table 3).

On the basis of the results of morphological features, physiological growth conditions, and biochemical identification tests of the isolate UV25, the identity of the bacterial isolate was determined as *Bacillus* sp. according to Bergey and Holt (1994).

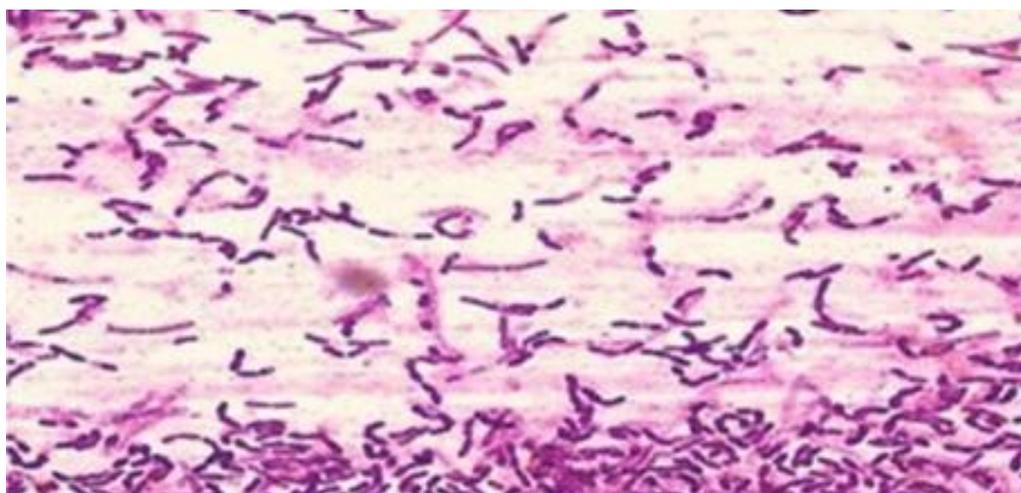


Fig. 3 Gram staining of laccase producing bacterial isolate UV25

Table 2. Morphological, physiological and biochemical characteristics of the bacterial isolate UV25

Morphological characteristics	UV25 strain	Physiological characteristics	UV25 strain	Biochemical tests	UV25 strain
Colony color	White	Spore formation	+	Lipase production	+
Shape	Circular	Sodium chloride	Growth at 1 to 5%	Gelatin liquefaction	+
Surface	Smooth	Growth at 4°C	-	Oxidase test	+
Gram's staining	Gram +ve	Growth at 55°C	-	Starch hydrolysis	+
Cell shape	Rods	Optimum growth temperature	37 °C	Casein hydrolysis	+
Endospore	+	Optimum pH	7.0	Citrate utilization	-
Motility	+			Indole production	-
				Catalase test	+
				Urease test	+

Table 3. Carbohydrate fermentation tests shown by the UV25 using HiCarbo™ kit

Part A		Part B		Part C	
Carbohydrate	UV25	Carbohydrate	UV25	Carbohydrate	UV25
Lactose	+	Inulin	+	Rhamnose	+
Xylose	+	Sodium gluconate	+	Cellobiose	+
Maltose	+	Glycerol	+	Melezitose	+

fructose	+	Salicin	+	a-Methyl-D- Mannoside	+
Dextrose	+	Dulcitol	+	Xylitol	+
Galactose	+	Inositol	+	ONPG (colourless)	-
Raffinose	+	Sorbitol	+	Esculin (black)	+
Trehalose	+	Mannitol	+	O..Arabinose	+
Melibiose	+	Adonitol	+	Citrate	+
Sucrose	+	Arabitol	+	Malonate (light green)	-
L-Arabinose	+	Erythritol	+	Sorbose	+
Mannose	+	a-Methyl-D- glycosidc	+	Congo Red	+

### 3.4. Laccase-producing bacterium from wood decaying soil sample origin

The comparison between different laccase-producing bacterium and their origin is shown in table 4. This study has reported a *Bacillus* sp. UV25 as a laccase-producing bacterium of wood decaying soil origin. Most other studies have explored soil from several other environmental samples. We found interest in this region because of decaying wood soil is a rich source for producing bacterium with ligninolytic potential that showing significant industrial application in variety of functions such as dye degradation, xenotoxic compound degradation, lignin degradation.

Some other investigators have also documented laccase production from *Bacillus* sp. harvested from soil (Srinivasan et al., 2019), *Bacillus* sp. strain BAB-4151 sequestered from a soap industry waste (Deepa et al., 2020), *Alcaligenes faecalis* XF1 sequestered from the green site (Mehandia et al., 2020), *Bacillus cereus* AKRC03 harvested from sludge samples collected from pulp and paper mill Lalkuan, Utrakhand, India (Kumar and Chandra, 2021), *Lysinibacillus macroides* LSO from Alexandria paper and pulp industry effluents (Abdelgalil et al., 2022), *Bacillus* sp. KC2 from marine environment (Asadia et al., 2020), and *B. amyloliquefaciens* from wastewater samples collected from the effluent of Cloverbrook Textile Company, Cairo, Egypt (El-Bendary et al., 2021).

Overall, this experiment led to the isolation of a potent laccase secreting bacterium from soil impregnated with decaying wood which was identified as *Bacillus* sp. UV25 on the basis of its morphological, physiological, and biochemical characteristics.

Table 4. Isolation of laccase-producing bacteria from natural sources and their comparison

S. No.	Source of isolation	Laccase producing Bacterial strain(s)	Substrate in the medium	References
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1.	Wood decaying soil	<i>Bacillus</i> sp.	Guaiacol	Present study
2.	Soil from Baghdad	<i>Bacillus cereus</i> B5	Syringaldazine	Allos and Hussein (2015)
3.	Contaminated sediments from a refinery, Bizerte coast, Tunisia	<i>Pseudomonas extremorientalis</i> BU118	2,6- DMP	Neifar et al. (2016)
4.	Soil and water from Golestan Salt Lake, Iran	<i>Aquisalibacillus elongates</i> SR-073	Guaiacol, 2,6- DMP and ABTS	Rezaei et al. (2017)
5.	Soil	<i>Anoxybacillus</i> sp. UARK- 01	Lignin	Thamir et al. (2017)
6.	Black liquor of a pulp paper factory, Jiangsu, P. R. China	<i>Bacillus subtilis</i> cjp3	ABTS	Qiao et al. (2017)
7.	Forest soil with decaying wood from Chandigarh and Himachal Pradesh	<i>Bacillus subrilis</i> DS	Guaiacol	Kumar et al. (2018)
8.	Sludge from the dumping site of Unnao Distilleries and Breweries, Uttar Pradesh, India	<i>Klebsiella pneumoniae</i> IITRCS01, <i>Salmonella enterica</i> IITRCS06, <i>Enterobacter aerogenes</i> IITRCS07, and <i>Enterobacter cloaceae</i> IITRCS11 (All are proteobacteria)	Guaiacol and Phenol red	Kumar and Chandra (2018)
9.	Soil from rhizosphere of potato crop, Sharkyia governorate, Egypt	<i>Bacillus halotolerans</i> N11	Guaiacol	Reda et al. (2018)
10.	Garden soil from Jubilant Organosys, Gajraula	<i>Pseudomonas fluorescens</i>	Guaiacol	Rajput and Mishra (2019)
11.	Soil sample from Tianjin, China.	<i>Bacillus amyloliquefaciens</i>	ABTS and 2,6- DMP	Wang et al. (2019)
12.	Wastewater from wastewater treatment plants	<i>Stenotrophomonas maltophila</i> BU16, <i>Pseudomonas mendocina</i> AEN16, and <i>Pseudomonas aeruginosa</i> DEJ16 ( $\gamma$ proteobacteria)	Guaiacol, anaphthol, syringaldazine, and ABTS	Unuofin et al. (2019)
13.	Soil from different trees in Bursa, Turkey	<i>Bacillus subtilis</i> LP2	Guaiacol	Yasar et al. (2019)
14.	Water and soil from Tattapani hot spring. Chhattisgarh, India	<i>Bacillus licheniformis</i> TPNRI and <i>B. licheniformis</i> TPNR6	Guaiacol	Sharma et al. (2020)

15.	Woody soil sample from Gupta Timbers, Uttar Pradesh, India	<i>Pseudomonas monteilii</i> and 7 proteobacterium.	Guaiacol	Rajput et al. (2020)
16.	Caspian Sea brine samples	<i>Bacillus atrophaeus</i> KC2	Guaiacol and syringaldazine	Asadia et al. (2020)
17.	Effluent of Textile Industry and Cloverbrook Textile Company, Cairo, Egypt	<i>Bacillus amyloliquefaciens</i> Al	Guaiacol	El-Bendary et al. (2020)
18.	Water samples from Sohma hot-sulfur spring. Gurugram, Haryana, India	<i>Brevibacillus agri</i>	Guaiacol and tannic acid	Panwar et al. (2020)
19.	Sludge from Paper Mill, Saharanpur, Uttar Pradesh, India	<i>Bacillus</i> sp. AKRC01	Guaiacol	Kumar et al. (2020)
20.	Soil samples containing sawdust and dairy effluents from the saw mills and Sangam dairy plant in Guntur district	<i>Enterobacter</i> sp. and <i>Bacillus</i> sp.	CuSO <sub>4</sub> , Guaiacol and ABTS	Jyothi et al. (2021)
21.	soil (rice paddy storage area soil) and The Yamuna River (Kalindi)	<i>Enterococcus</i> sp.	methyl orange and tannic acid	Patra and Gupta (2024)

## CONCLUSION

In this work, a novel bacterial strain which secreted extracellular laccase in the culture filtrate was sequestered by the plate assay using nutrient agar supplemented with guaiacol as substrate. A Total of 56 laccase producing bacterial isolates were obtained from soil sample of using agar medium containing 0.1 % Guaiacol. Guaiacol produces a reddish brown color around the colony, making it a sensitive substrate for screening bacterial isolates that produce extracellular laccase. Among isolate, UV25 demonstrated the highest laccase activity of 250.04 IU/L on quantitative screening by using modified Horikoshi's medium supplemented with Guaiacol as production media. Morphological and biochemical studies reveal that UV25 isolate showing high laccase activity was belongs to *Bacillus* sp. and, it displayed diversity and capability to utilize various biochemicals. The results obtained in the present study indicated that *Bacillus* sp. is a potential strain for laccase production can be exploited in biotechnological applications.

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## Conflicts of Interest

The authors declare no conflict of interest.

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