Research Article

Screening, Isolation and Biochemical Characterization of Laccase Producing Bacteria from Water for Industrial Xenobiotics Degradation and Evaluation of Antimicrobial Activity

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ABSTRACT

The screening, isolation and biochemical characterization of Laccase producing bacteria from soil (rice paddy storage area soil) and The Yamuna River (Kalindi Kunj) was carried out for evaluation of degradation of xenobiotics to solve environmental issues and determination of anti-microbial property. Since all researchers are mainly concentrating on fungal laccases. For isolation the sample was spread on nutrient agar media containing methyl orange was used. The primary screening was carried out using methyl orange and secondary screening by using tannic acid which form brown colour zone. While secondary screening two isoforms of laccase producing isolates was observed one (L1) show higher activity at higher temperature range (27-40℃) and other (L2) at lower temperature range (2-6℃). Optimization of culture conditions for laccase producing bacteria broth. Highly laccase producing bacterial isolates were examined for morphological and biochemical characterization according to Bergey's manual of systematic Bacteriology. The isolates were identified as Enterococcus sp. This study evaluates the potential of Laccase synthesized by Enterococcus sp. to degrade three synthetic dyes, dialysis assembly for L2 enzyme after and before dialysis and inhibit growth of S. typhi. To increase Laccase production inducers (0.2mM CuSO4.7H2O) was added in fermentation media (Nutrient broth) for four days. Enzyme activity was measured at 420nm using 1mM tannic acid. Maximum activity of laccase L1 (9.83U/ml) and L2 (8.51U/ml) was measured at day four. The dialysis assembly of laccase after on the basis of maximum laccase activity of LP-2 is 8.25U/ml for 5minute, then it's followed by 6.72U/ml for 3minutes. The purified laccase showed a decolourization potential of very good results on all applied dyes on table-3. However, optimum decolourization three-day efficiency ware observed average on safranine L1 (8.641, 14.81 & 32.51) and L2 (8.04, 4.5226 & 7.537) followed by indanthrone blue L1 (2.68, 24.83 & 13.08) and L2 (20.84, 7.63 & 20.8445). The decolourization efficiency on malachite green was L1 (1.546, 4.123 & 25.25) and L2 (6.9, 5.96 & 19.205) respectively. The zone of inhibition by Laccase was measured as 24mm.

Keywords: Laccase, Xenobiotics, Tannic acid, Antimicrobial and Fermentation.

INTRODUCTION

Laccase (Benzenediol: oxygen oxidoreductases) are a versatile oxidoreductase enzyme, are also called as "BLUE ENZYMES" because they are multinuclear copper containing enzymes[1]. Laccases are having the capability to oxidize a wide range of phenolic and nonphenolic compounds by converting oxygen molecule to water on concomitant four-electron reduction[2]. Laccases are abundantly present in various fungi and higher plants. 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 is usually hindered due to high fermentation period, low laccase yield and also their applicability only under mesophilic and acidic reaction condition.

Majority of the industrial operations are carried out in extreme conditions, i.e., higher temperature, pH and high salt concentration. Fungal laccase generally fails to work in these extreme environments[3,4]. Moreover, bacterial laccases have some additional advantages because of their cost-effective use in industrial applications, which include broad substrate specificity, enzyme production in a short time, thermostability and easy to clone and express in the host with suitable manipulation[5]. Bacterial laccases are useful in pulp and paper bio-bleaching, degradation of dyes, textile industry, pollutant degradation and biosensor development[6]. Laccase is a substrate-specific enzyme and in general, it oxidizes a wide range of substrates such as phenolic compounds, phenylpropanoids, azo dyes and indigo dyes[7]. Methoxy substituted Phenolic compounds such as hydroquinone and catechol are good substrates for the majority of laccases; moreover, phenolic compounds, *syringaldazine*, guaiacol and DMP (2,4-di-methoxy phenol) are commonly used substrates[8].

There are two types of laccases, true laccase and false laccase. Laccase which shows activity with tyrosine is called false laccase and that which does not show activity is called true laccase. Till date, blue laccase is the most studied one in comparison to yellow or white laccase[9]. First, bacterial laccase from rice rhizosphere was isolated in the year 1993, known as *Azospirillum lipoferum*[10]. Laccase producing bacteria are of mainly Gram positive. e.g., Bacillus, *Geobacillus,* Streptomyces, *Rhodo coccus*, *Staphylococcus*, *Azospirillum, Lysini bacillus* and *Aquisali bacillus*[11]. However, some Gram-negative bacteria like Pseudomonas, Enterobacter, Enterococcus, Delfia, Proteobacterium and *Alteromonas* are also able to secrete laccase[12].

MATERIALS AND METHODS

Collection of samples from contaminated sites

The sample was collected from the sites where high probability of Laccase producing bacteria. Since Laccase is a lignin degrading enzyme, so first sample was collected from the rice paddy storage area soil. According to report of centre for science and environment issued in 2019, The Yamuna River (Kalindi Kunj) was polluted due to discharge of contaminated effluent from textile industries into the water bodies, so high probability of bacterial species which degrade phenolic compounds i.e., the second sample was collected from The Yamuna River. The isolates were identified previously by Morphological and biochemical tests, and maintained on cetrimide agar medium as a selective medium, these isolates were prepared for screening experiments. The isolates were screened for laccase production according to method described by[13].

Screening of isolates bacteria

Primary screening

LB media with methyl orange used for primary screening which was decolorized by bacterial colony which produce laccase. Three colony from The Yamuna River sample plate and one colony from soil sample which was Further streaked on LB with methyl orange.LB media containing 0.125g Peptone,0.05g Yeast extract, 0.125g NaCl, 0.5g Agar and 25ml of distill water. Since methyl orange has Azo bond so can be oxidized by laccase. were previously grown on MSM with methyl orange agar and formation the clear zone around the its growth were transferred to the surface of MSM agar medium then the isolates were incubated at 30°C for 24 hrs. Clear zone around the colony was an indication of laccase production. The radius of a clear zone was measured using electronic ruler in mm.

Secondary screening (Quantitative screening) Preparation of bacterial inoculum suspension

LB media with tannic acid used for secondary screening (Quantitative screening) which was decolorized by bacterial colony which produce laccase. The three colony from plate of W_1 was picked through loop and streaked on quadrant 1, 2,4 respectively on NAM plate consist of tannic acid.

LB media containing 0.125g Peptone,0.05g Yeast extract, 0.125g NaCl, 0.5g Agar and 25ml of distill water. Since tannic acid has Azo bond so can be oxidized by laccase. were previously grown on MSM with tannic acid agar and formation the clear zone around the its growth were transferred to the surface of MSM agar medium then the isolates were incubated at 30°C for 24 hrs. Clear zone around the colony was an indication of laccase production. The radius of a clear zone was measured using electronic ruler in mm.

Morphological and Biochemical characterization of laccase producing bacterial isolates

High laccase producing bacterial isolates (LP-1 & LP-2) were examined for morphological and biochemical characteristics with reference to Bergey's Manual of Systematic Bacteriology. Gram, s staining and cell morphology of the isolated (LP-1 & LP-2) strains were performed according to standard protocol. Further, the isolates (LP-1 & LP-2) were subjected to biochemical tests like Starch hydrolysis, Urease, Lipase, oxidase, catalase, nitrate reduction, Sorbitol, De-mannitol, Indole production, methyl red, Voges Proskauer, Hydrogen Sulphide, Citrate utilization and Sugar fermentation.

Optimum Conditions for Laccase Production Effect of fermentation media

The influence of fermentation media on the production of laccase was tested by cultivating of laccase producing bacteria broth. isolate in different culture media include (MSM with Nutrient broth, Yeast extract media, Starch media). Erlenmeyer culture bottle (50ml) containing 25ml of each tested medium in duplicates were sterilized and inoculated with 1% of overnight culture of the isolate. Flasks were incubated in shaker incubator (150 rpm) for 24 hr at 37 ºC. After the incubation, supernatant from each flask were filtered by centrifugation and the filtrate was taken for the determination the enzyme activity, protein concentration and the specific activity[12].

Effect of temperature

Twenty-five ml of optimum fermentation medium was prepared in (250 ml) Erlenmeyer culture bottle, pH was adjusted to 7.0. These flasks were sterilized by autoclaving at 121˚C for 15 min., The tubes were inoculated with 100µl of Bacteria broth overnight culture of the isolate *Enterococcus sp*. Laccase production was achieved at different temperatures (4, 25, 37and 60). After sterilization, then incubated at pH 7.5 for 24hr[14]. After the incubation, supernatant was taken from each tube for the determination the enzyme activity, protein concentration and the specific activity with the help of spectrophotometer at 600nm.

Effect of pH value

To determine the influence of the initial pH value of the culture medium on enzyme production, Erlenmeyer flasks (250 ml) containing 25 ml of the selected fermentation medium were adjusted using 0.1N HCl or 0.1N NaOH to give different pH values ranging from 3 to 9 (3, 5, 7, 9 and blank), These flasks were sterilized by autoclaving at 121˚C for 15 min., The tubes were inoculated with 100µl of Bacteria broth overnight culture of the isolate *Enterococcus sp*. Then the culture medium was inoculated with the selected isolate and incubated in at 30ºC for 24hour[15]. After the incubation, protein concentration and the specific activity were measured with the help of spectrophotometer at 600nm.

Sub-merged fermentation production of laccase enzyme

The laccase enzyme production prepares a 25ml of NAM media above his composition 0.125g Peptone, 0.05g yeast extract, 0.125g NaCl, 2% agar, then this media Autoclave for 30minutes at 121°C. add inoculate the bacteria from the plate with the help of loop. It was placed in incubator at 37°C for 5 days. Add ammonium sulphate precipitation (100% stock of ammonium sulphate143.35g/200ml, Buffer Used: 1mM Sodium acetate buffer (pH= 4.5-5) Buffer composition: 1mM Sodium acetate buffer = Sodium acetate + Glacial acetic acid + Distilled water (https://www.encorbio.com/protocols/AM-SO4.htm). The stock was prepared by dissolving 143,35g of ammonium sulphate in 1mM sodium acetate buffer at 4°C. The ammonium sulphate was added into the fermentation broth taken from stock to achieve desired concentration of ammonium sulphate to achieve desired precipitation. The whole process was carried out into cold condition to avoid denaturation of protein. At 75% saturation protein was precipitated known as "salting out" and centrifuge at 10,000g for 40 minutes at 4°C and pellet stored at 2°C.

Screening for novel laccase producing bacteria

Isolation and screening were carried out by using the method described by[15]. 10grams of collected sample was transferred to 100 ml of nutrient broth supplemented with 0.2mM $CuSO_4$+ $7H_2O$ in 250 ml Erlenmeyer flask and incubated at 37°C for 48hrs on a rotary shaker(150rpm). By using standard serial dilution plate technique bacterial colonies were isolated from nutrient agar medium containing 0.2mM CuSO4 + $7H_2O$ and the plates were incubated at 37°C for 48 hrs.

Bacterial isolates were primarily screened for laccase production by adding 2-3 drops of 1mM tannic acid laccase substrate to the bacterial isolates. Green colour colonies were identified as laccase producing isolates. These colonies were isolated on to nutrient agar medium containing 0.2mM $CuSO_4$+$7H_2O$. Bacterial isolates were labelled as LP-1, LP-2. Further all the bacterial isolates were subjected for secondary screening for secondary screening, a loop full of bacterial suspension was inoculated in 50 ml of sterile LB broth supplemented with 0.01% CuSO4 and incubated at 37°C at 125 rpm[16]. Laccase activity was checked daily by tannic acid assay method.

Laccase Activity Assay (tannic acid Assay)

Laccase activity was measured using Tannic acid substrate for Laccase. The reactive mixture consists of 1.5 ml sodium acetate buffer (1 mM, pH 5.0), 1.5 ml tannic acid assay (0.5 mM) and 1.5 ml cell free supernatant. The absorbance was measured at 420nm, and 420nm (LP-1, LP-2) using UV/Visible Spectrophotometer[17]. One unit of enzyme activity was defined as 1 micro mole of tannic acid oxidized per minute. All the experiments on laccase activity were performed in triplicates.

Dialysis

The membrane was activated by boiling in EDTA solution for 30 minutes for five times. NaOH was used to dissolved EDTA in distilled water. As the membrane was activated the enzyme was filled into the membrane using pipette and tie up using rubber bands. Then it was placed into dialysis assembly. Dialysis was carried out against 100mM sodium acetate buffer. The buffer was changed after each 2-4h for 24 h. After 24h the membrane was placed into 6mM sucrose to remove access buffer. The whole process was carried out at 4°C (L2 laccase producing enzyme).

Degradation of Xenobiotic

Partial purified laccase was used for solving popular environmental issues such as Pesticide (Chlorpyrifos) contamination and removal of textile dye. Toxic dye used in experiment: Malachite green, safranine, Indanthrene blue (fabric dye). Malachite green- wavelength used 614nm. Safranine- wavelength used 519nm Indanthrene blue- wavelength used 614nm. Dye decolourization experiment was carried out for three days and each day percentage decolourization was absorbance was at respective wavelengths.

Antibacterial activity

Antibacterial activity of laccase and *S. typhi* strain was isolated from rotten fruit. Media composition: 2% agar, peptone, NaCl, yeast extract Incubation period: 24 h at 37°C. Antibiotic used: Ciproflaxin, this would be use as an alternative to antibiotic in future with certain modification to improve the activity of enzyme against bacteria to reduce harmful effect of antibiotic and against antibiotic resistance in bacteria. produced by laccase against the pathogenic test organisms *S. typhi* was evaluated by agar well diffusion method[18].

RESULTS AND DISCUSSION

Screening of *Enterococcus sp*. isolates for laccase production

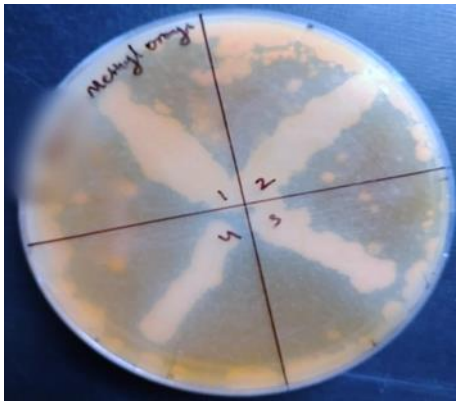
Primary screening using methyl orange solid medium

A simple method of screening was followed to determine laccase producing bacteria on MSM with methyl orange agar medium. The efficiency of bacterial isolates was compared by estimating the ratio between the colour less zone diameter around bacterial cultures and the growth zone. Among all *Enterococcus* sp. isolates were screened, among them fifteen isolates were laccase producer, whenever clear zone around the MSM with methyl orange plate well was formed. The clear zones ratio of isolates was number of zones. The isolates were further screening to select an active *Enterococcus sp*. isolate for laccase production[19] reported the ability of *Enterococcus sp*. to decolorize and degrade methyl orange at concentration up to 500 mg/l. LB media with methyl orange used for primary screening which was decolorized by bacterial colony which produce laccase. Three colony from The Yamuna River sample plate and one colony from soil sample which was

Further streaked on LB with methyl orange. Since methyl orange has Azo bond so can be oxidized by laccase. The spread plate method is a technique to plate a liquid sample containing bacteria so that the bacteria are easy to count and isolate. A successful spread plate will have a countable number of isolated bacteria colonies evenly distributed on the plate.

Streaking plate method

Quadrant 1,4 and 2 has colony picked from primary screening sample1 and 3 has from sample 2, least decolorization was observed in 1 quadrant and minimum in 3 quadrants so was eliminated from experiment. Further 2 and 4 quadrant colony were chosen for secondary screening. Q4 colony was designated as L1 (b) and L2 (a) as show the figure number -1.



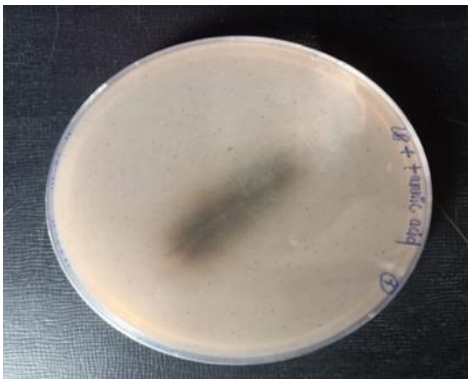
A

B

Figure-1: (A and B): L1, L2 Quadrant streaking method

Secondary screening (Quantitative screening)

On Sec. Screening two different bacterial strain producing two isoforms of laccase was found in L1 and L2. L1 laccase show strong zone at temperature greater than 27-35℃ and L2 show strong zone at 4-10℃. Secondary screening was carried out using 1% (2mM) Tannic acid. Tannic acid having phenolic compound so can be degraded by laccase into glucose and gallic acid which form brown color zones Streaking is a microbiology technique for isolating a pure strain from a single type of microbe, most often bacteria. A streak plate involves the progressive dilution of an inoculum of bacteria or yeast over the surface of solidified agar medium in a Petri dish. The result is that some of the colonies on the plate grow well separated from each other. The aim of the procedure is to obtain single isolated pure colonies on figure-2.



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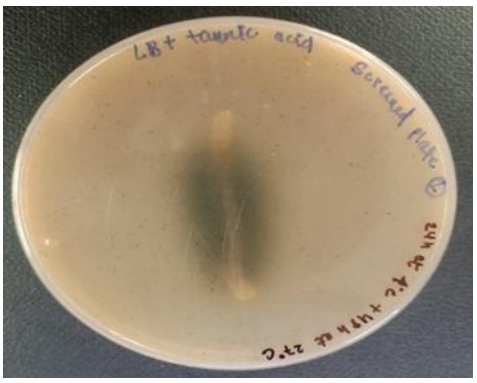


B

Figure-2: (A) L1 having colony from Q4 and (B) L2 having colony from Q2

Table 1: Biochemical and morphological characterization of the bacterial isolates (LP-1 & LP-2).

Sl. No.	Biochemical/ Morphological test	Bacterial isolate L-1	Bacterial isolate L-2
1.	Urease	+	-
2.	Lipase	+	-
3.	Catalase	+	+
4.	Nitrate reduction	+	+
5.	Indole Production	-	-
6.	Methyl Red	+	+
7.	Voges Proskauer's	+	+
8.	Citrate utilization	+	-
9.	Starch Hydrolysis	-	-
10.	Glucose	+	+
11.	Maltose	+	+
12.	Lactose	+	-
13.	Sucrose	+	+
14.	Mannitol	+	+
15.	Endospore	-	+
16.	Motility	-	+
17.	Gram staining	-	-

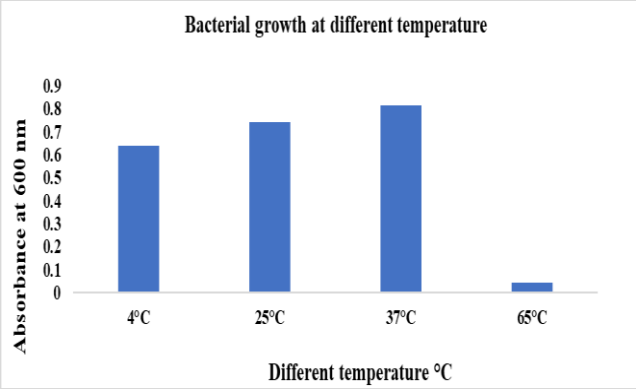
Morphological and biochemical identification laccase producing bacteria by ABIS online Software

Bacterial isolates, LP-1 and LP-2 were selected on the basis of ABIS online software, both bacterial isolates (LP-1 & LP-2) were tested for morphological and biochemical characterization as per Bergey's manual of Systematic Bacteriology. The results were shown in (Table.1). On the basis of cultural and biochemical characteristics, the bacterial isolates (LP-1 & LP-2) were identified as *Enterococcus sp.* Respectively.

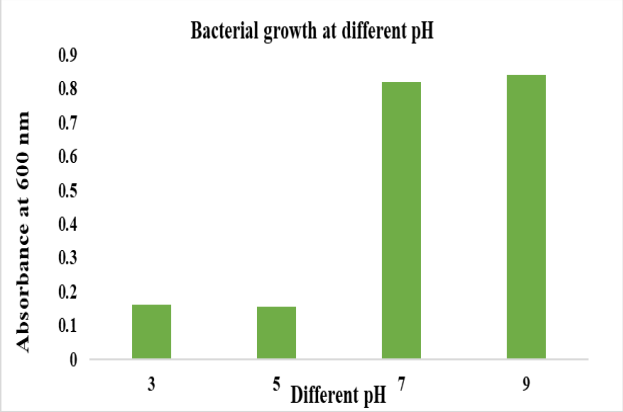
The biochemical test of L1, L2 is used for identification of bacteria using ABIS online software which show that bacteria belong to "*Enterococcus avium*, *Enterococcus malodorous*.

Optimization of culture conditions for laccase producing bacteria.

Growth optimization L2 Bacterial broth, the highest laccase producing bacteria growth was observed at 37ºC (0.813) while is followed bacteria growth was observed at 25ºC (0.741). (Graph: 1). Highest laccase producing bacteria growth was observed at pH 9 (0.839) while lowest laccase producing bacteria growth was found at pH 5 (0.155), (graph: 2). A gradual increase in the enzyme activity from 24 h was observed. After 24 h of incubation time, a decrease in the trend of enzyme activity was observed. Overall, the highest bacterial growth was observed at 24 h.



Graph 1: Effect of temperature on laccase activity.



Graph 2: Effect of pH on laccase activity.

Laccase activity test

Selected 2 isolates were subjected to secondary screening by laccase production assay in LB broth supplemented with 1% fructose and 0.01% $CuSO_4$ and incubated at 30°C at 125 rpm. Laccase activity was checked daily by Tannic acid assay method. The results were shown in Table-2. Out of 2 bacterial isolates, LP-1 was selected on the basis of maximum laccase activity of 9.84 U/ml. For testing of laccase activity 800µl 1mM tannic acid was used as a substrate along with 1700µl sodium acetate buffer and 500µl broth. The absorbance was measured at 420 nm. Extinction coefficient of tannic acid at 420 nm for 36000.

$$\text{Laccase Activity (V/M}_1) = \frac{A_{420} \times V_1 \times 10^6}{t \times €_0 \times V_2}$$

A= Absorbance at 420nm

V_1 = Total volume of Rx^n mixture (ml)

T = Total incubation period (min)

$€_0$ = Molar extinction coefficient tannic acid (36000)

V_2 = Volume of enzyme used (ml)

Table 2: Quantitative screening of laccase activity from bacterial isolates: LP-1 and LP-2.

Incubation time	Laccase activity of LP-1 (U/ml)	Laccase activity of LP-2 (U/ml)
Day 1	3.42 ± 0.02	2.2 ± 0.02
Day 2	5.81 ± 0.01	3.51 ± 0.01
Day 3	8.63 ± 0.01	5.72 ± 0.02
Day 4	9.84 ± 0.03	8.51 ± 0.01

Dialysis

The dialysis was carried out to remove excess salt from the sample by using the diffusion principle. It was carried out against 100mM sodium acetate buffer for 24 hours. After this, the protein obtained was partially purified and it was resuspended in 10mM sodium acetate buffer at 4. After dialysis the laccase activity was measured at 420nm at 1-, 3-, and 5-minute time interval. For testing of laccase activity 20µl 1mM tannic acid was used as a substrate along with 2960µl sodium acetate buffer and 20µl pure enzyme.

The absorbance was measured at 420 nm. Laccase activity was calculated using below mentioned formula. Extinction coefficient of tannic acid at 420 nm is 36000.

- Laccase Activity (V/M_1) = $\frac{A_{420} \times V_1 \times 10^6}{t \times €_0 \times V_2}$

A= Absorbance at 420nm

V_1 = Total volume of Rx^n mixture (ml)

T = Total incubation period (min)

$€_0$ = Molar extinction coefficient (36000)

V_2 = Volume of enzyme used (ml)

Table 3: Dialysis assembly of laccase activity from laccase enzyme after and before dialysis: LP-2.

Incubation time in (minutes)	Dialysis assembly before laccase activity of L-2 (U/ml)	Dialysis assembly after laccase activity of L-2 (U/ml)
1	5.52 ± 0.03	5.75 ± 0.01
3	6.32 ± 0.01	6.72 ± 0.01
5	6.89 ± 0.02	8.25 ± 0.02

Selected 1 isolates were subjected to dialysis assembly by laccase production assay in dialysis enzyme. Laccase activity was checked after and before by Tannic acid assay method. The results were shown in Table-3. Bacterial isolates, LP-2 was selected after on the basis of maximum laccase activity of 8.25U/ml for 5minute, then it's followed by LP-2 is 6.72U/ml for 3minutes.

Degradation of xenobiotics:

Dye decolourization experiment was carried out for three days and each day percentage decolourization was calculated using below mentioned formula and absorbance was calculated at respective wavelengths.

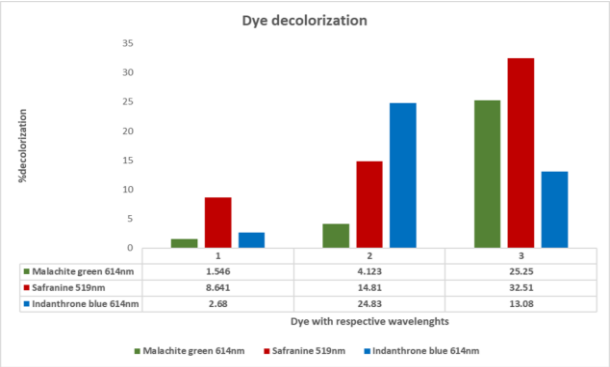
$$\text{Percentage of decolourization} = \frac{A_0 - A_t}{A_0} \times 100$$

A_0 = Absorbance at time (t) = 0

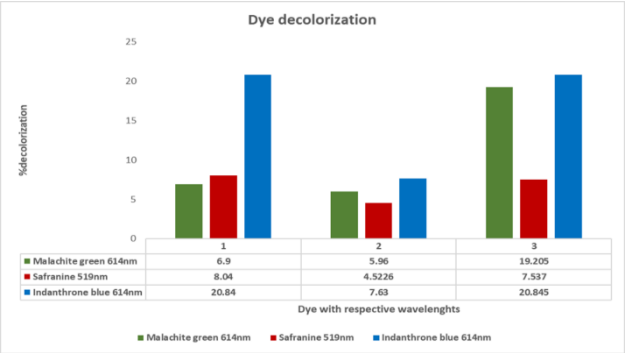
A_t = Absorbance at certain time (t) = t

The purified laccase showed a decolourization potential of very good results on all applied dyes on graph- 3&4. However, optimum decolourization three-day efficiency ware observed average on safranine L1 (8.641, 14.81 & 32.51) and L2 (8.04, 4.5226 & 7.537) followed by indanthrone blue L1 (2.68, 24.83 & 13.08) and L2 (20.84, 7.63 & 20.8445). The decolourization efficiency on malachite green was L1 (1.546, 4.123 & 25.25) and L2 (6.9, 5.96 & 19.205) respectively. The maximal dye decolourization potential of ***Enterococcus sp***. laccase is an indication for its prospects for industrial applications. *Enterococcus amyloliquefaciens* can produce laccase with efficient dye decolourization prospect[20]. Similarly, *Oudemansiella canarii* produced laccase with about 55% decolourization efficiency on safranine[21]. The application of laccase as an azo-reductase is topical[22,23]. Consequently, enzyme application for the decolourization of industrial dyes portrays an efficient treatment and management system on diverse dye effluents in industrial discharge that impairs the quality of the environment[24].

Graph 3 & 4. Potential of the purified laccase from *Enterococcus sp*. decolourize Malachite Green, Indanthrone blue and safranine all three-day Comparison and average percentage of dye decolorization by L1 and L2. Overall, the laccase produced by *Enterococcus sp*. effectively degraded the azo dyes and holds good potential for application in the industrial sector.



Graph 3: L1 Comparison of Degradation of xenobiotics dry decolorization all three days.



Graph 4: L2 Comparison of degradation OD xenobiotics dry decolorization all three days.

Antibacterial activity

Antibacterial activity of laccase was tested against *S. typhi.* This strain was isolated from rotten fruit, by agar well diffusion method. No zone of inhibition observed in negative control. The diameter of laccase bacterial broth is 24mm[25] reported that, the Antibiotic used: Ciproflaxin. Zone of inhibition of enzyme: 34mm Antibiotic used: Ciproflaxin show the table-4.

Table 4: Antimicrobial activity against *S. typhi.*

Different sample	Zone of inhibition (mm)
Negative control/Blank	0
Positive control/Antibiotic	34mm
Laccase producing enzyme	24mm

CONCLUSION

Bacterial laccase is more effective compare to fungal laccases with more tolerant to extreme condition and can be used for solving major environmental issues. This enzyme also used as a weapon against pathogenic strain of *S. typhi* to fight anti biotic resistance which is the major issue arises due to misuse of antibiotics Total 2 Laccase producing bacterial isolates were obtained from Noida district Yamuna River (Kalindi Kunj) water using agar medium containing 0.2 mM $CuSO_4$ and 2 mM Guaiacol. Guaiacol is a responsive substrate for screening the laccase producing bacterial isolates. In this study, 2 potent Laccase producers showing high laccase activity (LP-1 & LP-2) were identified as *Enterococcus sp*. according to morphological

and biochemical characterization. using Tannic acid as substrate showed maximum laccase activity of LP-1 & LP-2 (9.84 & 8.51) and stability at temperature 37°C, pH 9, and 6mM substrate concentration in 30 min incubation period. Partially purified laccase efficiently degraded of safranine, Malachite green and of indanthrene blue was degraded after 3 days of incubation at 37ºC It also has proficient antibacterial activity against *S. typhi,* antibiotic is 34mm this is the first report on enzyme laccase is 24mm and its use for decolorization of synthetic dyes. Therefore, this strain can be used to decolorize and detoxify the industrial effluents and help in wastewater treatment.

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