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Research Article

ISOLATION AND CHARACTERIZATION OF LIGNIN DEGRADING BACTERIA

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ABSTRACT

Ligninolytic bacteria has remained an interesting research area from decades. Optimization of enzyme extraction from fungi is a tedious task, thus, focus has been shifted to bacteria which possess the capability to degrade lignin. This research assessed few bacterial isolates for ligninolytic and peroxidase properties for the utilization and degradation of lignin into its constituents. Five bacterial isolates were studied for their potentiality to degrade lignin using peroxidase enzyme. The isolates were sub-cultured numerous times to achieve pure strain. The screened colonies for lignin degradation were subjected to morphological and biochemical tests. Each of the isolate was evaluated for lignin degradation, total enzyme activity and total protein. Isolate 3 (C_3) was found to be the potential bacterium in terms of growth rate (lignin degradation), activity (30.4 U/mL), and protein concentration (32mg/mL). Further C_3 was identified as *Arthrobacter* sp. E147 which belongs to the family Micrococcaceae by 16S rRNA sequencing. The protein from each isolate was analyzed by SDS-PAGE. The potentiality of the aforementioned bacterial isolate for its industrial applications is abundant since there is a lack of information on organisms with such potential.

Keywords: Lignin, Peroxidase, Guaiacol, Catechol, Vanillin.

1. INTRODUCTION

Lignin is an aromatic polymer with a high heterogenic complex that is linked to a carbohydrate by an ester linkage. It has a lignocellulose content of nearly 30% [1]. Lignin is made up of three types of phenolic compounds: guaiacyl (G), syringyl (S), and hydroxyphenyl (H), are polymerized by cross-linking [2]. Many microorganisms are capable of fully degrading lignin; however, some microorganisms only partially degrade lignin because they lack the enzymatic capacity to attack all of the polymer's structural elements. In the biosynthesis of lignin as lignocelluloses and non-conjugated lignin, monomeric replacements such as phenyl propylene, coniferyl alcohol, coumaryl alcohol, and sinapyl alcohol are used. Since lignin is a part of the plant cell wall and has a complex structure, its degradation is considerably difficult.

Peroxidase (EC 1.11.1.x), also known as peroxide reductase, is a biological catalyst made up of large groups of enzymes. Peroxidases are oxidoreductases enzymes that can catalyze a reaction using a free radical, allowing certain compounds to be converted to their oxidized or polymerized forms. Peroxidases can detach hydrogen atoms from alcoholic groups, resulting in the formation of water and oxidized phenolic compounds when combined with hydrogen peroxide. Glycoproteins with iron ions, prosthetic units, and a carbohydrate moiety make up these peroxidases. The oxidative process can generate peroxidases in fungi, bacteria, plants, and animals, but the efficiency of fungi in degrading compounds is higher than that of bacteria. Lignin peroxidase (EC 1.11.1.14) is an enzyme that oxidizes lignin by degrading the non-phenolic portion of it using peroxide as a catalyst. Peroxidase uses hydrogen peroxide (H_2O_2) as a substrate, is a relatively gentle oxidizing agent. Unlike other oxidizing reagents, hydrogen peroxide is known as a highly beneficial reagent because its yields only water and oxygen as by-products and no inorganic salts. As a result, it is regarded as the ultimate "green" reagent. Around 47% of its weight is given as an oxidant, which is significantly higher than any other oxidant [3].

Guaiacol, also known as o-methoxyphenol is an aromatic compound that is yellow-orange in colour. It has high viscosity and is usually oily in nature. Wood creosote or guaiacum and thermal degradation of benzoic acid derivatives from peat and malt smoke are used to make guaiacol. It functions as a superoxide radical scavenger. Hence, it is used to treat dental pulp as an antiseptic, expectorant, local anaesthetic,

analgesic, and sedative [3]. At a broad range of pH (between 3-9) and temperatures (between 30-80°C), peroxidase can effectively split hydrogen peroxide into its byproduct. On phenolic substrates, the optimum temperature for peroxidase operation is 15-25°C or 25-30°C, with a transformation time of 1-3 h. At a pH range of 6.0-7.5, the process performance improves. Peroxidases isolated from bacteria can break lignin to vanillin and other derivatives.

The chemical composition of lignin is extremely complicated. As a result, it causes a slew of issues. While lignin and its degraded products have a lot of asymmetric centers, they do not have any optical operation. Fungi and bacteria are among the microorganisms that majorly degrade lignin. Lignin may also be degraded by microbes such as cyanobacteria and actinomycetes. The metabolism of indigenous micro-organism attributes to the degradation of lignin. Examples of lignin-degrading bacteria are *Paenibacillus, Aneurinibacillusaneurinilyticus,* and *Bacillus sp. Streptomyces; Streptomyces antibioticus, S. griseus, S. coelicolor, S. cyaneus,* and *S. lavendulae* are examples of lignin-degrading actinomycetes. *Stropharia rugosoannulata, Agaricus bisporus, Ganoderma applanatum,* and *Heterobasidion annosum* are some of the examples of lignin-degrading fungi [3]. Objective of the current study was to perform Screening, isolation and characterization of lignin degrading bacteria from lignin induced soil.

2. MATERIAL AND METHODS

2.1. Isolation of microorganism

Soil samples were collected from PWD road, Kailashahar, Tripura, India and Hawamahal Society, Ankleshwar, Gujarat, India. About 1 gram of lignin enriched soil was serially diluted by suspending it in 10 mL of sterile distilled water and the suspension was swirled. This was considered as the initial test tube and labelled as 10^0. Eight other test tubes were taken for serial dilution and labelled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} respectively. Each of these test tubes was filled with 9 mL of sterile distilled water. From the initial test tube (labelled as10°), 1 mL was taken and re-suspended in the tube labelled as 10^{-1} giving a dilution of 10 times. From this 10^{-1} tube again 1mL was taken and re-suspended in the next test tube labelled 10^{-2} to give a dilution of 100 times. This was carried out for all the successive test tubes until the highest dilution was reached in the test tube labelled 10^{-4}, were plated on an LB agar plate containing lignin. A total of six plates were made with different concentration of lignin namely, 0.1%, 0.2%, 0.5%, 1%, 1.5% and 2% respectively. The plates were incubated at 35 ± 2°C for a period of 24-48 h. All the isolates obtained were sub cultured numerous times on LB agar plates containing 0.5% of lignin to achieve pure strains of each isolate.

2.2. Purification of microbial culture

The purification of the isolated bacterial strains (C_1-C_5) was performed using different microbiological procedures. Inoculum of 24 h cultures on the LB Agar medium containing 0.5% of lignin was inoculated into conical flasks containing mineral salt media with 0.5% lignin and was incubated 35±2°C for a period of 24 to 72 h. After incubation sample was taken and centrifuged at 10000 rpm for 10 minutes. Further, pellet was then re-suspended in fresh mineral salt media containing 0.5% lignin. This procedure was repeated numerous times in order to confirm the homogeneity of this culture.

2.3. Identification of microbial strain

Total genomic DNA was isolated by N-Cetyl - N, N, N-trimethyl-ammonium bromide (CTAB) method. A loop full of culture was taken in a tube containing CTAB buffer and crushed with Micro pestle. 20 µL of β-Mercaptoethanol was added to the crushed culture. It was then incubated in water bath at 60°C for 60 minutes. 500µL of PCI was added to the tube and centrifuged at 10000 rpm for 10 min. After centrifugation, the supernatant was transferred to a fresh tube. Equal volumes of Chloroform: Isoamyl alcohol (24:1) was added to the tube and it was centrifuged at 10000 rpm for 10 min. The aqueous phase was removed and taken in a sterile micro-centrifuge tube. Isopropyl alcohol was added up to full and incubated at -20°C for 1hour. It was centrifuged at 10000 rpm for 10 min. Pellet was washed in 500µL of 70% ethanol and centrifuged at 10000 rpm for 10 min. Pellet was dried and dissolved in 20µL sterile distilled water. The quantity of the isolated DNA was checked in UV-VIS spectrophotometer (Vivaspec Biophotometer, Germany). From the stock 1µL DNA was mixed with 49µL sterile distilled water to get 50 times dilution. The A260/A280 ratio was recorded to check the purity of DNA preparation.

PCR amplification of 16S region was done in 20µL of reaction mixture containing PCR buffer, 1X (Kappa SA); $MgCl_2$, 3 mM dNTP mix, 0.25 mM Taq DNA

polymerase, 0.05 U primer 1 picomole and template DNA 50 ng. Sterile nuclease free water is used as negative control. The PCR reaction was performed outin a thermal cycler with an initial denaturing step of 94°C for 2 min, 35 cycles of denaturing at 94°C for 50 s, annealing at 55°C for 30 s and an elongation at 72 for 1 min 30 sec and a final cycleof extra elongation at 72 for 6 min. Samples were separated on 1.5% agarose gel.

2.4. Cultural and biochemical test of the isolated strain

The isolated pure strain which utilized lignin as the sole source of carbon was subjected to various biochemical tests such as Oxidase activity, Catalase test, Indole production, Citrate utilization, Methyl Red-Voges Proskauer (MR-VP), Gelatin hydrolysis (liquefaction) and Starch hydrolysis.

Morphological characteristics of the isolated micro-organisms were determined by performing various cultural tests. The cell characteristics and nature of the culture in nutrient-agar and LB- agar media were observed at different incubation periods.

2.5. Analysis of substrates and products

To determine the fate of the accumulated metabolites, the experiments were continued for two days and samples were removed after 24-72 h incubation period. The sample was centrifuged and the supernatant was collected. The pH of the supernatant was adjusted to pH 2 by adding 100 µL of 0.5 N HCl. Then, 800 µL of ethyl acetate was added and the tubes were vortexed for 1 minute. The aqueous phase (catechol, vanillin, pyruvate and gallic acid) was extracted with ethyl acetate (1:5 v/v) and further extracts were evaporated. The dried sample was suspended in ethyl acetate for TLC analysis.

2.6. Analysis of metabolites by Thin Layer Chromatography

TLC was carried out on silica plates (silica gel pre-coated, 6 x 3.7 cm dimension with 2.0 mm thickness) by spotting the ethyl acetate extracts on the plate along with the authentic substrate (lignin) and metabolite (vanillin, catechol, gallic acid and pyruvate). The plates were kept in the mobile phase i.e., a mixture of toluene, ethyl acetate and toluene in the ratio of 4:5:1 using the method of Caroline Nehvonen [4]. The metabolites were visualized by exposing the plates to UV light and iodine vapours.

2.7. Extraction of peroxidase

The 24 h grown bacterial cells were harvested by centrifugation at 10000 rpm for 10 minutes at 4°C. The cells were then resuspended in citrate buffer pH 6.0 and subjected for sonication (vibra cell VC8130 watts) in cold conditions. The bacterial lysate was centrifuged at 10,000 rpm for 20 minutes at 4°C and the cell free lysate was used as the source of the enzyme.

2.8. Peroxidase assay

After implicating a few modifications, the activity of peroxidase was assayed by using H_2O_2 as a substrate. The activity of peroxidase was measured using Chance's and Machly's method in terms of micromoles of tetra guaiacol formation.

2.9. Protein estimation

Protein content was estimated by the Lowry method using bovine serum albumin as standard [5].

2.10. Electrophoresis

The protein extracts were resolved by electrophoresis on SDS PAGE using Lammelli's method [6]. The separating gel consisted of 12% (w/v) acrylamide, 0.1% (w/v) SDS<0.04% TEMED, 0.375 M Tris-HCl, pH 8.8 and 0.1% (w/v) ammonium persulfate. The gels were 10X16 cm and 1.0 mm thick, 100 µg sample of total soluble extracts was applied per lane. The samples were mixed with an equal volume of 2x SDS-PAGE gel loading buffer (100 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 5% β ME and 2% bromophenol blue) and the proteins were denatured in boiling water bath for 5 minutes. The electrophoresis was carried out in 1x electrophoresis buffer (25 mM Tris-HCL, pH 8.0, 250 mM glycine and 0.1% SDS) at 100 V until the bromophenol blue dye 5 mm away from the bottom of the gel. Protein was stained by using Coomassie brilliant blue G-250.

3. RESULT AND DISCUSSION

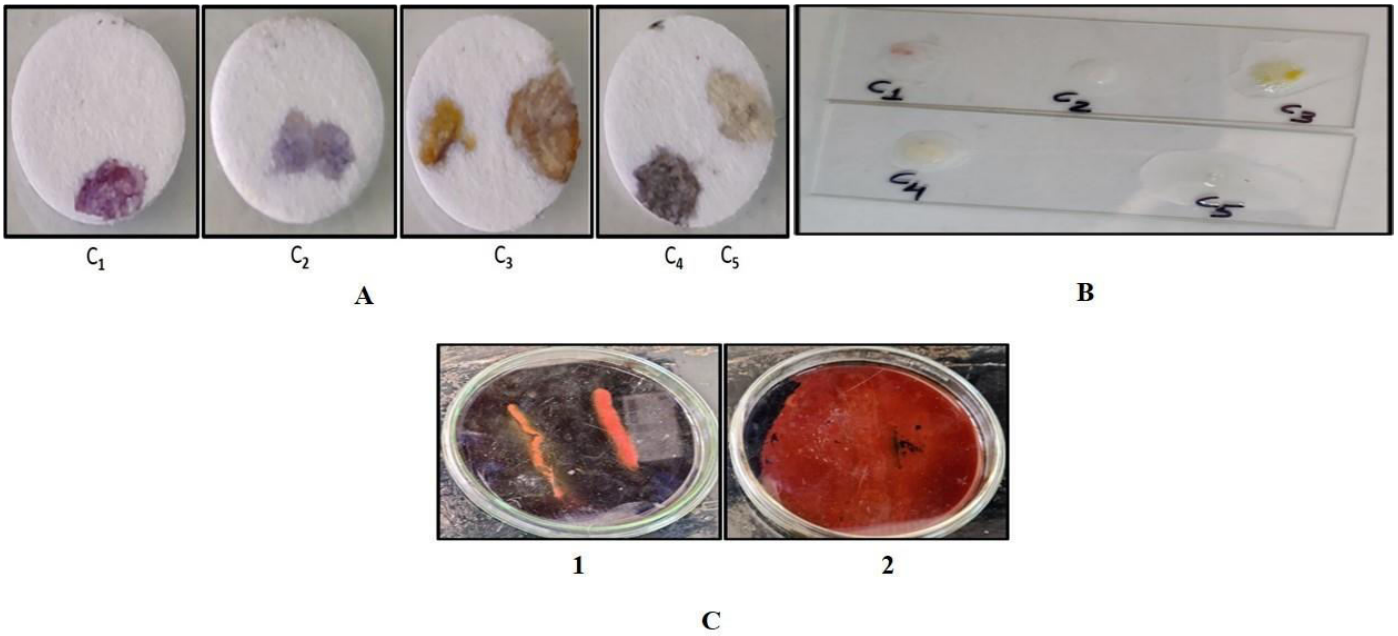
3.1. Isolation and purification of the Micro-organisms

Five bacteria possessing the potential for lignin degradation were isolated from lignin enriched soil samples. The bacterial strains capable of producing the enzyme were screened according to methods of Bergey's manual of determinative bacteriology [7] and further confirmed by analyzing the morphological and biochemical properties. 0.5%lignin concentration was found to be optimum for the growth of the bacteria.

After the incubation period, good growth of the bacteria and also colour change of the LB agar was observed which is the indication of efficient degradation of lignin to different intermediates of the biochemical synthetic pathway (Catechol, Vanillin etc.). All the isolates were confirmed as lignin degraders by conformational testing through a change in the colour of the LB (Figs. 1 and 2). Based on efficient degradation and peroxidase activity, Isolate C_3 was identified as *Arthrobacter sp.* E147 by 16S rRNA sequencing [8].

However, studies have also shown other bacteria to possess ligninolytic potential. Thirty ligninolytic bacterial isolates were collected from Tyhume river from which two bacterial strains ($T1CS3^D$ and $T2BW3^1$) showed enormous potential in lignin degradation and peroxidase production [9]. In addition, many *Bacillus* strain are also capable to degrade lignin [10, 11]. Four species from twenty isolates were studied for the production of peroxidase namely, *Pantoeadispersa, Pseudomons aeruginosa B. subtilis* and *S. aureus*. Among the four, *P. aeruginosa* species (PA3) was found to be the efficient bacteria to produce peroxidase [12].

Further, the growth was also analysed in MSM media without lignin where it showed no growth of microorganisms, thereby confirming that the bacterial isolates were able to use lignin as the sole source of carbon (Fig. 1).



A: Oxidase Test
C_1 - Positive
C_2 - Positive
C_3 - Negative
C_4 - Positive
C_5 - Negative

B: Catalase test
C_1- Positive
C_2- Positive
C_3- Positive
C_4- Positive
C_5- Positive
C_3-Arthrobacter sp. E147

C: Starch hydrolysis test
Plate 1- Isolate 3 and 5
Plate 2: Isolate 4

Fig. 1: Biochemical Tests

3.2. Identification of the Microbial Strain

3.2.1. Biochemical tests

Morphological characteristics of the isolated micro-organisms were determined by performing various cultural tests. The cell characteristics and nature of the culture on Nutrient agar and LB-agar media were observed at different incubation period. The isolated pure strain which utilized lignin as the sole source of carbon was subjected to various tests such as Oxidase activity, Catalase activity, Indole production, Methyl Red-Voges Proskauer (MR-VP), Citrate utilization, Gelatin hydrolysis (liquefaction) and Starch hydrolysis to determine the morphological, physiological and biochemical characteristics of the organisms (Fig. 1 and

Table 1). To be able to determine the gram character and endospore formation of the isolates, gram staining and endospore staining were also carried out in replications (Table 2, 3 and Figs. 2 and 3). Colony characteristics of the isolates are given in table 4.

Table 1: Results of various biochemical tests carried out on lignin degrading bacteria *Arthrobacter* sp. E147

Biochemical test	Result
Oxidase test	Negative
Catalase test	Positive
Indole test	Negative
Methyl Red (MR) test	Negative
Voges Proskauer (VP) test	Negative
Citrate Utilization test	Positive
Gelatin liquefaction test	Negative
Starch hydrolysis test	Positive

Table 2: Gram characteristics of the isolates and *Arthrobacter* sp. E147

Isolate	Shape	Gram character
1	Rods	Negative
2	Rods	Positive
Arthrobacter sp. E147	Rods	Positive
4	Rods	Positive
5	Rods	Positive

Table 3: Endospore staining results of the bacterial isolates

Isolate	Endospore
1	Absent
2	Absent
Arthrobacter sp. E147	Absent
4	Absent
5	Absent

Table 4: Colony Characteristics (Morphological Test)

Colony/Isolate	1	2	*Arthrobacter* sp. E147	4	5
Margin	Entire	Entire	Entire	Filiform	Entire
Shape/form	Round	Round	Round	Irregular	Round
Elevation	Raised	Flat	Raised	Flat	Raised
Surface Texture	Smooth	Smooth	Mucoid	Rough	Smooth
Consistency	Butter like	Butter like	Viscous	Dry	Butter like
Opacity	Opaque	Opaque	Opaque	Opaque	Transparent
Chromogenesis	Creamy pink	Creamy white	Yellow	Off white	Colourless

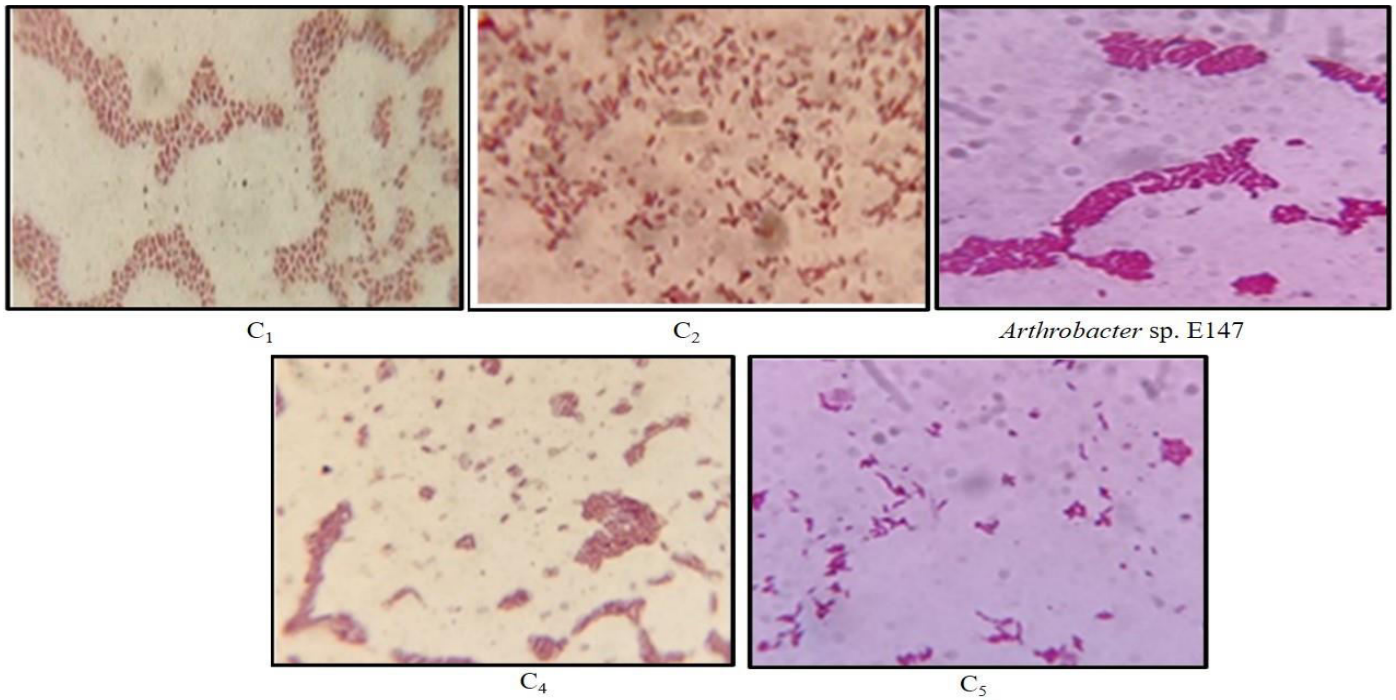


Fig. 2: Microscopic view of bacterial Isolate-1, Isolate-2, *Arthrobacter* sp. E147, Isolate-4 and Isolate-5

3.2.2. *Identification of strain*

16S rRNA sequencing and phylogenetic analysis for Isolate 3 were done and the strain was identified as *Arthrobacter sp*. strain E147 and the DNA of 16SrRNA gene band was found to be 250bp (Figs. 4 and 5). Similar study was reported for a strain of *Providencia* sp. involved in the lignin biodegradation process. The strain was identified using 16S rRNA sequencing method and DNA of 16S rRNA gene band was found to be 1500 bp in size [12].

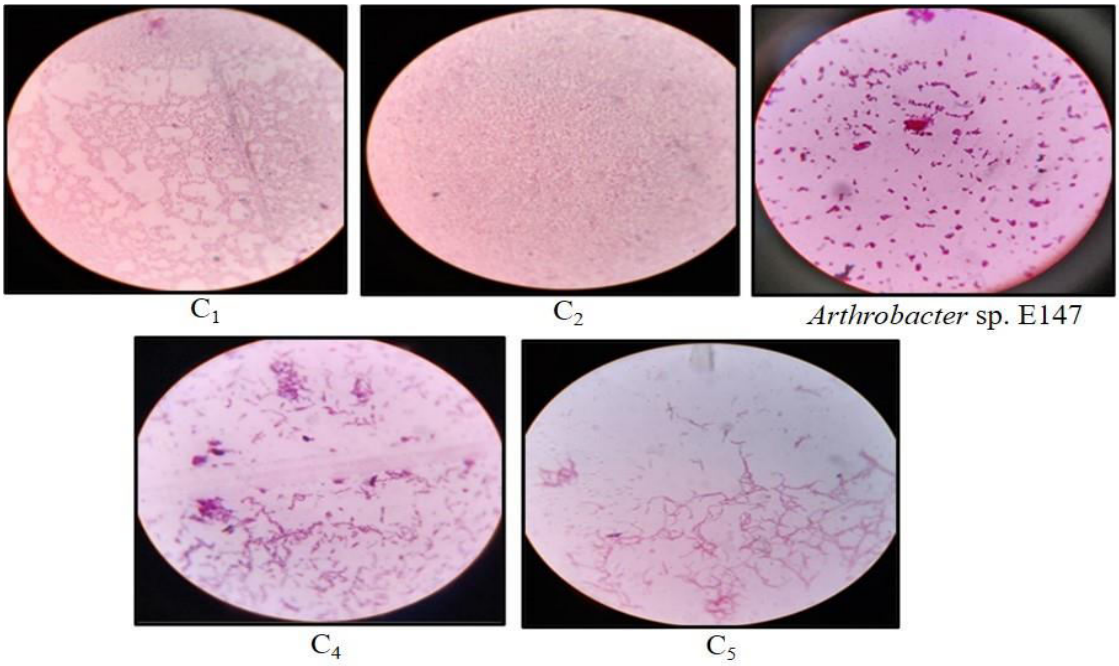


Fig. 3: Microscopic view of endospore staining of bacterial Isolate-1, Isolate-2, *Arthrobacter* sp. E147, Isolate-4, Isolate-5 respectively

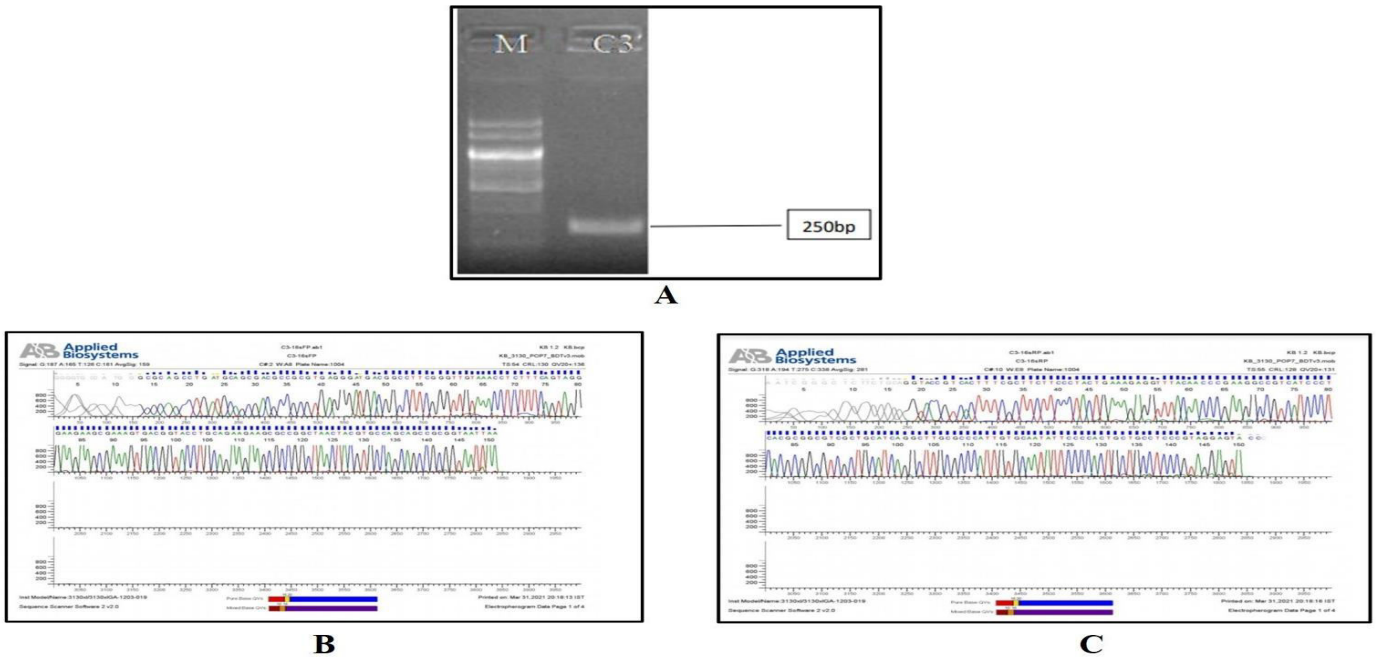
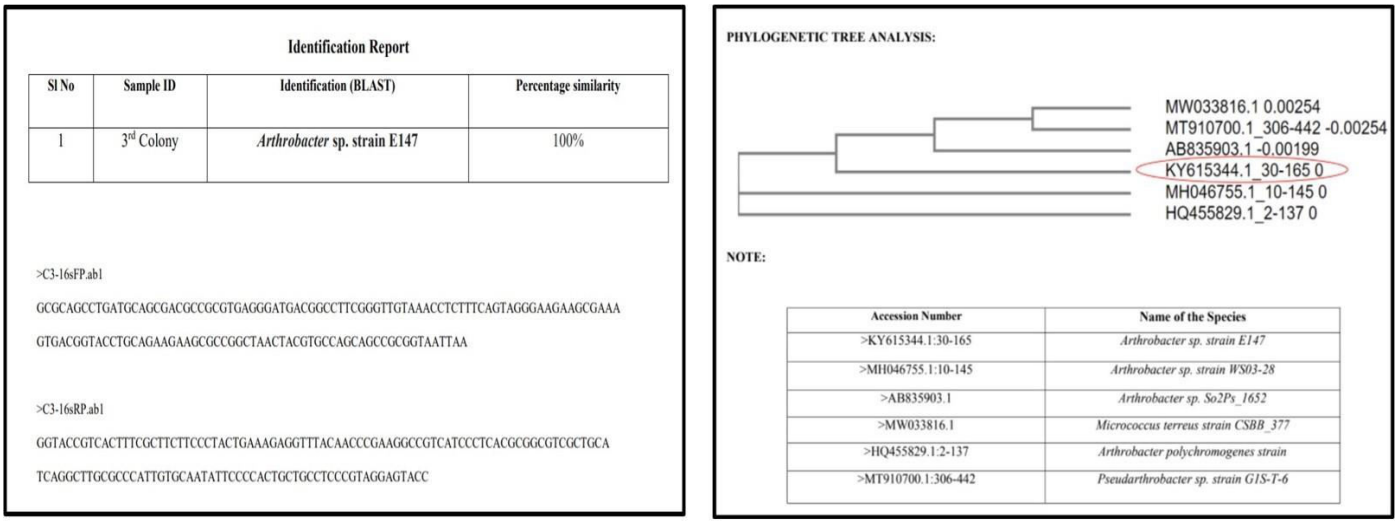


Fig. 4: (A) Agarose gel electrophoresis for amplification of 16S rRNA gene of *Arthrobacter* sp. E147 bacteria on (1.5% Agarose Gel); (B)16S Forward Primer Sequencing; (C) 16S Reverse Primer Sequencing



Identification Report

Sl No	Sample ID	Identification (BLAST)	Percentage similarity
1	3rd Colony	*Arthrobacter* sp. strain E147	100%

>C3-16sFP.ab1

GCGCAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAAGAAGCGAAA

GTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATTAA

>C3-16sRP.ab1

GGTACCGTCACTTTCGCTTCTTCCCTACTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGCA

TCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTACC

PHYLOGENETIC TREE ANALYSIS:

NOTE:

Accession Number	Name of the Species
>KY615344.1:30-165	*Arthrobacter sp. strain E147*
>MH046755.1:10-145	*Arthrobacter sp. strain WS03-28*
>AB835903.1	*Arthrobacter sp. So2Ps_1652*
>MW033816.1	*Micrococcus terreus strain CSBB_377*
>HQ455829.1:2-137	*Arthrobacter polychromogenes strain*
>MT910700.1:306-442	*Pseudarthrobacter sp. strain G1S-T-6*

A B

Fig. 5: (A) 16S rRNA Sequencing of *Arthrobacter* sp. E147; (B) Phylogenetic analysis of *Arthrobacter* sp. E147.

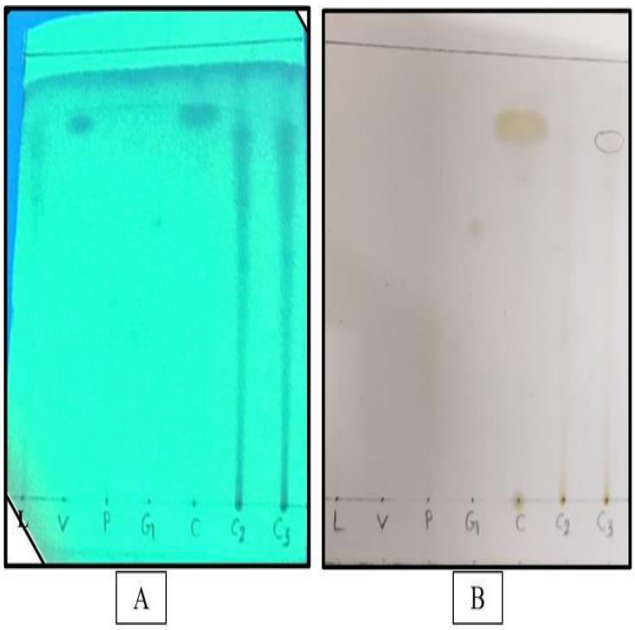
3.3. Analysis of Substrates and Products by Thin Layer Chromatography

The isolates, after extraction with ethyl acetate were analyzed by TLC [4]. The samples were incubated at different time intervals and the results obtained showed different intensity of band at different incubation period. On exposure to UV light and iodine vapours catechol, gallic acid and vanillin bands were observed. A corresponding band to catechol was observed in *Arthrobacter sp*. strain E147 whichindicates that lignin is broken down to one of its derivatives i.e., catechol. The method was carried in duplicates and the same result was obtained. In plate A, Isolate 2 (C_2) and *Arthrobacter sp.* strain E147showed bands adjacent to the catechol (Fig. 6 and Table 5, and in plate B *Arthrobacter sp.* strain E147 and Isolate 5 (I_5) also showed the bands adjacent to catechol (Fig. 7 and Table 6).

Table 5: R_f values

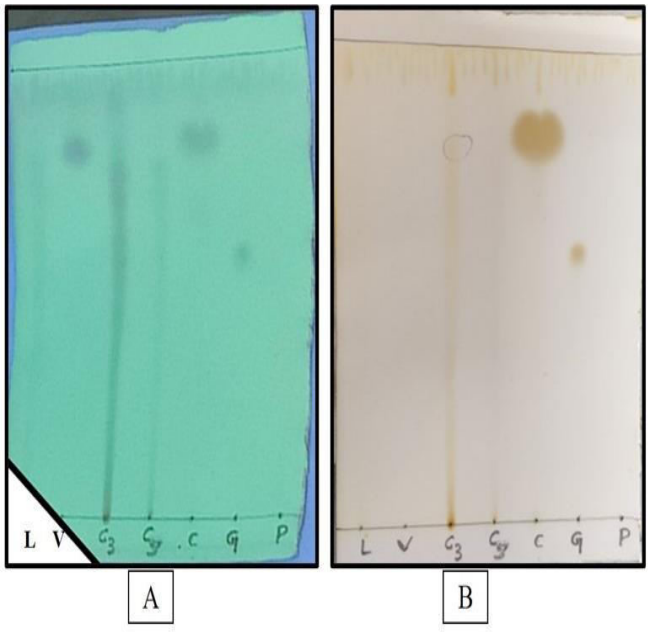
Substrate	Resolution factor (R_f value)
Pyruvate	0.54
Vanillin	0.92
Gallic acid	0.69
Catechol	0.86
Isolate 2	0.86
Arthrobacter sp. E147	0.86

However, TLC carried out in another study reported the degradation of vanillin to vanillyl alcohol by the microorganism. A standard of vanillyl alcohol had been included in TLC analyses. The compound formed during cultivation showed potent similarities with vanillyl alcohol (standard), which suggested that vanillyl alcohol is a possible biodegraded product from vanillin by the microbial strain [4].



*(**A**) TLC plate exposed under UV light (**B**) TLC plate exposed to iodine vapours. **L**-Lignin, **V**-Vanillin, **G**-Gallic Acid, **C**-Catechol, C_2-Isolate 2 and C_3-Arthrobacter sp. E147.*

Fig. 6: Thin Layer Chromatography (TLC)



A) TLC plate exposed under UV light, B) TLC plate exposed to iodine vapours. L-Lignin, V-Vanillin, G-Gallic Acid, C- Catechol, C_5 - Isolate 5 and C_3-Arthrobacter sp. E147

Fig. 7: Thin Layer Chromatography (TLC)

Table 6: R_f values

Substrate	Resolution factor (R_f value)
Pyruvate	0.47
Vanillin	0.86
Gallic acid	0.6
Catechol	0.81
Isolate 2	0.81
Arthrobacter sp. E147	0.81

3.4. Protein estimation

The protein content of enzyme sample was estimated for each isolate (C_1-C_5). *Arthrobacter sp.* strain E147 showed the highest OD or absorbance at 660 nm (1.32 mg/mL) followed by C_2 (1.2 mg/ml), C_5 (0.6 mg/ml), C_4 (0.54 mg/mL) and C_1 (0.48 mg/mL) (Fig. 8). Bradford method was used to determine quantitative protein concentrations by calculating optical density at 595 nm using bovine serum albumin (BSA) as a reference [13]. Lowry method was also used to quantify protein, using bovine serum albumin as a reference [5].

3.5. Assay of enzyme

The activity of peroxidase was measured spectro-photometrically at 470 nm using guaiacol as a substrate [12]. Guaiacol, in the reaction mixture, oxidizes and forms tetraguaiacol. Tetraguaiacol is a brown complex and its intensity is measured at 470nm. The activity of *Arthrobacter sp.* strain E147 was found to be the highest followed by C_2. *Arthrobacter sp.* strain E147 again showed the highest peroxidase activity with modification of assay conditions of about 50 U/mL which is followed by C_2 with 45.1 U/mL. C_5 showed 30.4 U/mL activity followed by C_1 with 20.3 U/mL while C_4 showed 11.3 U/mL (Fig. 9).

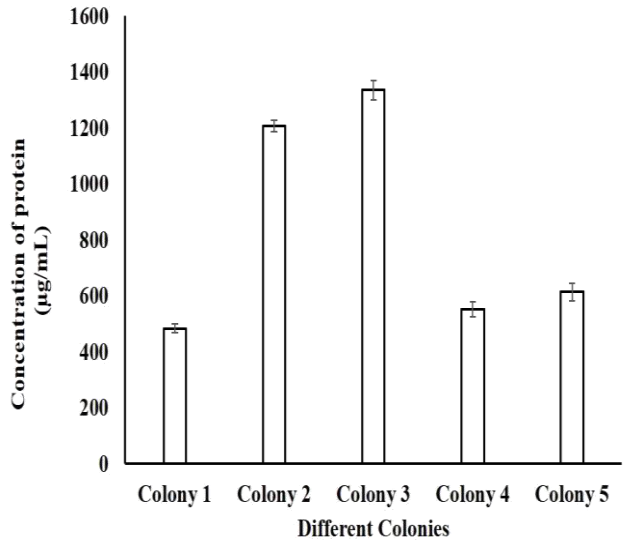


Fig. 8: Analysis of total protein content from different colonies/isolates (Colony 3-*Arthrobacter* sp. E147)

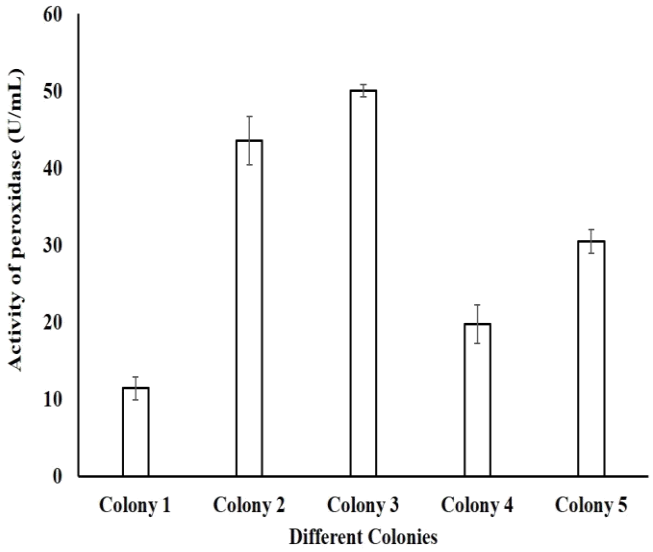


Fig. 9: Analysis of total peroxidase activity from different colonies/isolates (Colony 3-*Arthrobacter* sp. E147)

The enzyme activity in *Urginea indica* and *Urginea polyphylla* was reported to be 0.0096 U/mL and around 0.0072 U/mL in *Urginea wightii* [14]. The activity of the peroxidase enzyme was measured as a shift in absorbance at 436 nm per minute/g fresh tissue. Twenty of the reported isolates from four separate species of *Pantoea dispersa*, *Pseudomonas aerogenosa*, *Bacillus subtilis*, and *Staphylococcus aureus* bacteria were analyzed for peroxidase production. The findings of the study revealed different levels of activity for the products. *Pseudomonas aerogenosa* P1 bacteria appeared to be the most successful among the rest of the bacterial isolates. Consistent results were also reported during peroxidase estimation in *Providencia spp.*, *Pseudomonas aerogenosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pantoea agglomerans* [14-19].

Further, *Arthrobacter sp.* strain E147 alone was estimated for the enzyme activity at different volumes (100 µL, 200 µL, 300 µL). The results obtained were recorded as follows: 124 U/mL for 300 µL, 101.5 U/mL for 200 µLand 90.2 U/mL for 100 µL (Fig. 10).

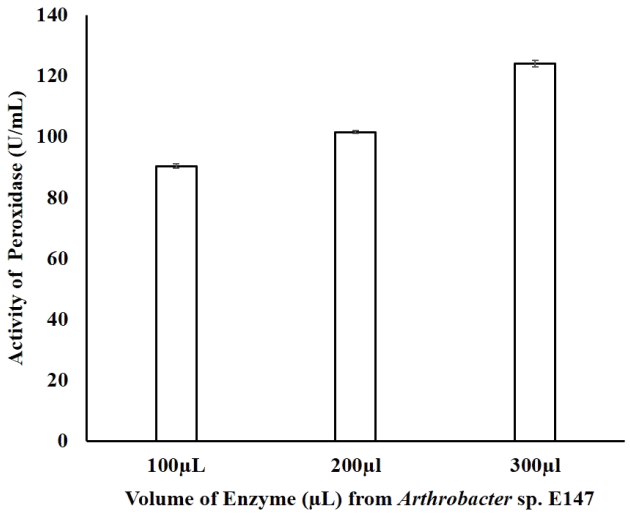
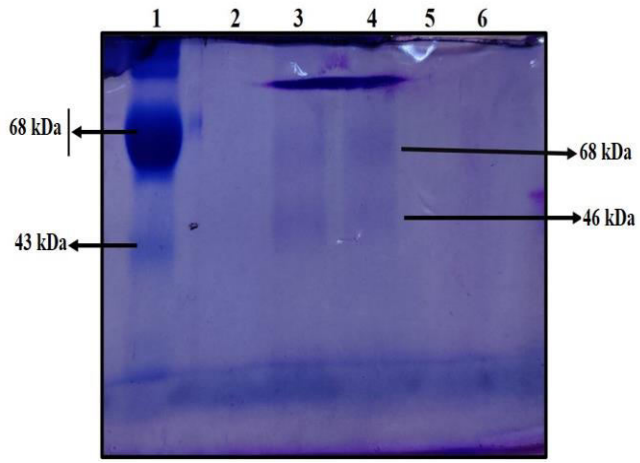


Fig. 10: Analysis of total peroxidase activity from *Arthrobacter* sp. E147 with different aliquots

3.6. Electrophoresis

SDS-PAGE was performed on crude culture filtrateusing a 12 percent polyacrylamide gel according to Laemmli [6]. Coomassie brilliant blue (0.04%) staining was used to detect protein lines. Standard molecular weight marker BSA with molecular weight 68 kDa and 43 kDa was loaded. The staining with Coomassie brilliant blue and destaining revealed C_2 and C_3 (*Arthrobacter sp.* strain E147) with two prominent bands of about 68 kDa and approximately 46 kDa. Isolate 2 and 3 showed a darker band pattern when compared to other isolates (Fig.11).

In a study, the enzyme's purity determined using SDS-PAGE, was reported as a single band on the gel with a molecular weight of 55 kDa in comparison to the normal molecular weight. This molecular weight falls within the spectrum of peroxidases found in a variety of foods, such as horseradish and oil palm leaves. The molecular weights of the above-mentioned and peroxidase isoenzymes from various sources range from 30,000 to 60,000 Dalton [20]. With guaiacol as a substrate, it was reported that the enzyme activity staining revealed a single band corresponding to the peroxidase activity position. Molecular weights of 43 kDa and 110 kDa were recorded previously for bacterial peroxidases purified from *Bacillus sp.* VUS [21] and *Acinetobacter calcoaceticus* NCIM 2890 [22].



Lane 1, BSA; Lane 2, Colony 1; Lane 3, Colony 2; Lane 4, Arthrobacter sp. E147; Lane 5, Colony 4; Lane 6, Colony 5.

Fig. 11: SDS-PAGE profile

4. CONCLUSION

Peroxidase is an important enzyme and it has a wide range of applications but there is always scope for peroxidase with better characteristics that shows several applications in diverse fields including pharmaceutical industries. It has been observed that peroxidase from a few generations of white-rot fungi can be used for degrading wastes due to their non-specific extracellular enzymatic system involved in lignin biodegradation [23]. Dye wastes are considered xenobiotics. Xenobiotics are the most problematic group of pollutants as they do not hold a good biodegradability profile. These synthetic

dyes are emitted into the industrial effluents which lead to the pollution of the environment. Microbes such as fungi and bacteria have emerged as an extremely valuable alternative to achieve biodegradation of hazardous pollutants [24]. Several peroxidases isolated from bacteria have been used for the decolorization of synthetic dyes. Extracellular peroxidases, isolated from ligninolytic microbes play a major role in the complete mineralization of xenobiotic compounds. PAHs are degraded and transformed by MnP and LiP in the presence of hydrogen peroxide, hence leading to the formation of less mutagenic and easily degradable quinones and hydroxylated derivative [25]. EDCs act as agonists or antagonists of hormones. EDCs act as hormone mimics and trick the body to recognize them as hormones while some EDCs block the naturally occurring hormones. Several classes of oxidative enzymes have shown the potential to efficiently remove EDCs that resist conventional wastewater treatment. EDC can also be oxidized by manganese peroxidase [26].

A new third-generation biosensor for H2O2 has been established by cross-linking HRP onto an electrode that is modified with multiwall carbon nanotubes [27]. However, purification and in-gel analysis would give insights to the potent source of the enzyme for further applications.

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Conflicts of interest

We declare that we have no conflicts of interest regarding the contents of this article.

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