Research Paper Chemistry

Extraction of Lignin Peroxidase Enzyme from Bacteria Isolated from the Mangrove Wood

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ABSTRACT

Total eleven bacterial isolates were isolated from a degraded mangrove wood. These bacterial were screened for lignin peroxidase enzyme. Isolate MW6 showed the production of lignin peroxidase enzyme. This bacterium was incubated for different hours and it the enzyme production was found to increase gradually. The lignin peroxidase enzyme producing microbial isolate was identified as Bacillus licheniformis with the help of 16S rRNA amplified gene sequences

KEYWORDS Lignin, degradation, Mangroves, enzyme, 16S rRNA

INTRODUCTION

Lignin is an essential part of the plant cell wall and is structurally complex. Isolating and studying various microorganisms which can degrade lignin is becoming important in recent years as the enzymes they find applications in paper-pulp and various other industries (Perez et al., 2002).

A perusal of literature indicates that there are many microbial species which have the ability to produce such enzymes (Ahmad et al., 2010). Though, fungi form the majority of these organisms and are known as a source of excellent lignin degrading enzymes, they are difficult to use in industrial applications owing to their structural complexities and stability issues (Atalla et al., 2010). Bacteria are preferred over fungi as they possess immense environmental adaptability and biochemical versatility (Bholay et al., 2012). So identification of bacteria having lignin oxidizing enzymes could be significantly important for industrial and environmental point of view. Considering this, the present study was undertaken.

MATERIALS AND METHODS

Collection mangrove wood samples

Degraded wood was collected from the mangrove ecosystem near Ratnagiri, Maharashtra. The collected wood sample was immediately brought to the laboratory for further processing.

Isolation of wood associated bacteria:

For the isolation of bacteria, rotten wood was cut in small pieces and crushed in to 10 ml sterile seawater. This mixture was further diluted (serial dilution 10-1 to 10-5) and plated homogenates on Zobell marine agar (ZMA). The plates were incubated at 30°C for 2-5 days for bacteria The bacterial isolates were purified on ZMA and maintained further on ZMA slants.

Screening of bacteria for lignin peroxidase enzyme

The isolated bacterial cultures (total eleven) were screened for Lignin Peroxidase production. For the same, the bacteria were inoculated in Lignin Peroxidase screening medium as described by Sivakami et al., (2012).

The plates were incubated for 7 days to observe the reddish brown colour change in the screening medium. The strain showing positive for lignin peroxidase enzyme was further taken for enzyme production by culturing it on the lignin producing culture media. The production medium was collected and centrifuged to collect the supernatant which was used as enzyme source for the assay procedures.

Microfiltration of culture supernatant

The cell free supernatant was then filtered through 0.3µm glass fiber filter using vacuum pressure to remove the suspended particles. The micro filtered sample was collected in a separate container and used further for analysis.

Lignin peroxidase Assay

To 1 ml of culture supernatant, 2 mM Vetryl alcohol, 0.27 mM H2O2 and 10 mM tartarate buffer (pH 3.0) were added and incubated for 30 mins. The colour change was read at 310 nm. The enzyme activity can be measured by amount of enzyme required to convert one unit of Vetryl alcohol into Vetryl aldehyde during the incubation period.

Identification of enzyme producing bacterium

The enzyme producing bacterium was identified by using 16S rRNA gene sequences.

DNA Extraction was carried out using HiPurA Bacterial Genomic DNA Purification Kit (Himedia, MB505) using the standard protocol. Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation A11454806498). The DNA was stored at -200C till further use.

The DNA isolated from bacteria was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler (T-Personal 48). The PCR reaction mix contained 2.5µl of 10X buffer, 1µl of each primer (Table 2), 2.5µl of 2.5mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1µl Template DNA and 8.5µl nuclease free water. The PCR amplification cycle consist of, a cycle of 5 min at 94°C; 35 cycles of 1min at 94°C, 1 min at 50°C, 2 min at 72°C; and additionally 1 cycle of 7 min at 72°C. The reagents used were procured from GeNei.

Gel electrophoresis was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The size obtained was approx. 850bp for partial 16srRNA region

The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of PCR product 519F - 5' CAGCAGCCGCGGTAATAC 3' sequencing primer was used.

The DNA sequences were analyzed using online BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Center for Biotechnology Information (NCBI).

RESULTS

There were total 11 bacteria were isolated and cultured from the degraded mangrove wood samples. The details of these bacteria are given in the Table 1.

Table 1: Bacterial culture isolates from degraded mangrove wood

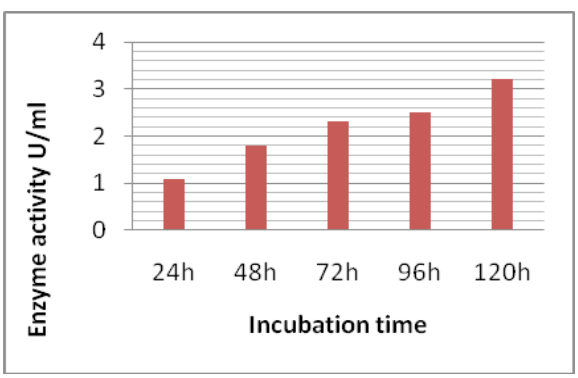
Culture code	Pigmentation	Colony morphology	Colony size
MW1	Cream	round	1-2mm
MW2	Off white	round	1-3mm
MW3	Light orange	round	Point
MW4	Colourless	round	3mm
MW5	Off white	round	3mm
MW6	Light brown	round	2mm
MW7	White	uneven	8mm
MW8	Light brown	round	4-5mm
MW9	Orange	round	4-5mm
MW10	Yellow	round	1-2mm
MW11	Yellow	round	3-4mm

Out of these eleven bacterial isolates, only one bacterium MW6 showed positive results for lignin peroxidase enzyme (LiP) in qualitative assay.

Lignin peroxidase activity

The enzyme activity of MW6 culture at different incubation (culture) time was estimated and presented in Fig. 1. The production of the enzyme in the broth was found to increase gradually. The maximum enzyme yield (3.2 U/ml) was observed at 120 h incubation.

Figure 1: Production of lignin peroxidase by MW6 at different incubation time



Bacterial identification using 16S rRNA gene sequence

The partial gene sequence of the amplified 16S rRNA gene of the extracted DNA from the isolate MW6 produced a 637 bp product (Fig.2).

Figure 2: Partial gene sequence of 637 bp of the bacterial isolate MW6

This amplified sequence of the 16S rRNA gene when analysed using BLASTn tool indicated that the isolate is from the genus Bacillus and most likely be a strain of Bacillus licheniformis as it showed 99% sequence similarity with many other Bacillus licheniformis strains which were already deposited in the nucleotide database (Table 2).

Table 2: Phylogenetic neighbours of the isolate MW6 based on partial 16S rRNA gene sequence

Description	**Ident**.	Accession
Bacillus licheniformis strain DSM 13 16S ribosomal RNA gene, complete sequence	99%	NR_118996.1
Bacillus licheniformis strain BCRC 11702 16S ribosomal RNA gene, partial sequence	99%	NR_116023.1
Bacillus licheniformis strain NBRC 12200 16S ribosomal RNA gene, partial sequence	99%	NR_113588.1
Bacillus licheniformis strain ATCC 14580 16S ribosomal RNA gene, complete sequence	99%	NR_074923.1

DISCUSSION

The results of the present study indicate that less explored areas like mangrove ecosystem could act as a hot spot to find novel microbes having the ability to produce bioactive compounds and secondary metabolites such as enzymes which can have industrial applications.

Lignin Peroxidase (EC 1.11.1.14) belongs to the family of oxidoreductases. Lignin Peroxidase (LiP) is an extracellular hemeprotein, dependent of H2O2, with an unusually high redox potential and low optimum pH. Due to their high redox potentials and their enlarged substrate range LiP have great potential for application in various industrial processes.

In the present study, the extracted enzyme from Bacillus licheniformis was found to have an activity of 3.2 IU. Though this is a promising activity but needs further purification as it is lower than reported by Patil (2014) who reported an enzyme activity of 4.2 IU from lignin peroxidase extracted from Bacillus megaterium.

Enzyme producer isolate was successfully identified using the amplified gene of 16S rRNA. Bandounas et al., (2011) isolated three novel bacterial isolates having the LiP production and identified then as Pandoraea norimbergensis LD001, Pseudomonas sp LD002 and Bacillus sp LD003 based on their 16S rRNA gene sequences. They reported that Bacillus sp LD003 was the best enzyme producer. So based on the results in the present study and earlier reports such as those by Raj et al., 2007, it is suggested that further studies to explore more lignin peroxidase producing strains should focus on bacteria from the genus Bacillus.

CONCLUSION

The present investigation highlights the lignin degrading activity of the enzyme produced by a Bacillus strain isolated from a degrading mangrove wood. We can also conclude that molecular marker like 16S rRNA gene sequence can be effectively used for the identification of the unknown microbes up to genus level.

REFERENCES

1. Ahmad, M., Taylor, C. R., Pink, D., Burton, K., Eastwood, D., Bending, G.D. and Bugg, T.D. (2010), "Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal lignin degraders". Molecular bioSystems, 6(5), 815-821.
2. Atalla, M.M., Zeinab, H.K., Eman, R.H., Amani, A.Y. and Abeer, A.A.E.A. (2010), "Screening of some marinederived fungal isolates for lignin degrad ing enzymes (LDEs) production". Agriculture and Biology Journal of North America, 1(4), 591-599.
3. Bandounas, L., Wierckx, N.J.P., Winde. J. H. de and Ruijssenaars, H.J. (2011), "Isolation and characterization of novel bacterial strains exhibiting ligninolyt ic potential". BMC Biotechnology, 11, 94.
4. Bholay, A.D., Borkhataria, V., Jadhav, U., Palekar, S., Dhalkari, V. and Nalawade, P.M. (2012), "Bacterial Lignin peroxidase: A tool for biolecching and biodegradation of industrial effluents". Universal Journal of Environmental Research Technology, 2(1), 58-64.
5. Patil, R. S. (2014), "Production and purification of lignin peroxidase from Bacillus megaterium and its application in bioremidation". CIBTech Journal of Microbiology, 3 (1), 22-28.

6. Perez, J., Munoz-Dorado, J., de la Rubia, T. And Martinez, J. (2002), "Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview". Int Microbiol , 5(2),53-63.
7. Raj, A., Reddy, M.M.K. and Chandra, R. (2007), "Identification of low molecular weight aromatic compounds by gas chromatography-mass spectrometry (GCMS) from kraft lignin degradation by three Bacillus sp.". Int Biodeter Biodeg, 59, 292-296.
8. Sivakami, V. Ramachandran, B., Srivathsan, J., Kesavaperumal, G., Smily, B., Mukesh Kumar D.J. (2012), "Production and optimization of laccase and lignin peroxidase by newly isolated Pleurotus ostreatus LIG 19". J. Microbiol. Biotech. Res., 2 (6), 875-881.