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Research Article

Isolation And Identification of Ligninolytic Fungi from South Gujarat Region

Riddhi S. Vashi*[1], Mayuri Dholaria[1]

[1]Department of Microbiology, Naran Lala College of Professional and Applied Sciences, Navsari, India

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***Corresponding author**
Riddhi S. Vashi,
Department of Microbiology, Naran Lala College of Professional and Applied Sciences, Navsari, India.
Email: riddhivashis777@gmail.com

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Abstract

Adequate amount of lignocellulosic biomass on earth features convincing feedstock material for various sustainable products such as bioplastics, biofuels and biomedical applications. However, delignification is necessary for converting complex material into valuable products. Among various pretreatment methods, biological delignification is particularly attractive due to its environmental friendliness. Ligninolytic fungi from Ascomycota and Basidiomycota are the primary target due to their secretion of oxidative enzymes like Laccase, LiP, and MnP, which initiate the degradation of its stable structure. Isolation, screening, and identification of potential ligninolytic fungi is an aim of this investigation. A total of 18 various fungal strains were isolated by using potato dextrose agar medium from 15 different collected environmental samples. Through quantitative and qualitative screening, three potential fungi labelled as F5, F8, and F9, were selected and identified as *Fusarium chlamydosporum* (PX482664), *Polyporus benetostus* (PQ348730), and *Trichoderma erinaceum* (PQ349085) using the ITS region-based molecular method. The Laccase activity of F-5, F-8, and F-9 were found to be 261.32 U/g, 434.29 U/g, and 207.67 U/g, respectively, while their LiP activity was determined to be 503.1 U/g, 430.5 U/g, and 330.6 U/g, respectively. However, no MnP activity was observed in all three fungal cultures.

Keywords: ligninolytic fungi; biological pretreatment; laccase; lignin peroxidase.

Introduction

Lignocellulosic biomass, a universal and renewable organic source which is primarily composed of cellulose, hemicellulose and recalcitrant aromatic heteropolymer lignin (Ragauskas *et al*., 2014; Salvachua *et al.,* 2015). It seems to be an important raw material for production of substitutes for petroleum-derived chemicals (Sanchez, 2009; Ragauskas *et al*., 2014; Salvachua *et al.,* 2015). However, presence of complex polyphenolic lignin having high molecular weight and ether linkages traps carbohydrates that hampers effective utilization of lignocellulosic biomass as carbon resources (Bugg *et al.,* 2011; Brown and Chang 2014; Mathews *et al.,* 2016). Efficient bioconversion of lignocellulose into value-added products, often necessitating pretreatment focusing on delignification for the effective utilization of cellulose and hemicellulose (Wang *et al.,* 2013; de Gonzalo *et al.,* 2016). Conventional pretreatment methods including physical, physicochemical, and chemical approaches (e.g., mechanical comminution, steam explosion, acid hydrolysis, oxidative delignification) are used to break down the complex lignocellulosic network to enhance the accessibility of cellulose and hemicellulose (Kumar *et al.,* 2009; Gilca *et al.,* 2014; Yang *et al.,* 2015). However,

requirements of expensive equipment, consumption of more energy and generation of toxic compounds makes these methods unsuitable. Alternatively, the biological pretreatment of lignocellulosic biomass through microorganisms and their enzymes, offers a more environmentally sustainable and economically viable strategy for enhancing enzymatic saccharification and producing bioenergic chemicals like ethanol and butanol (Huang *et al.,* 2013; Sharma *et al.,* 2017). Biodelignification is predominantly carried out by ligninolytic microorganisms notably white rot fungi (WRF), firmicutes and gamma proteobacteria which are regarded as the most efficient natural degraders of lignin. Extracellular oxidative enzymes like copper containing laccase (Lac - EC 1.10.3.2), heme containing manganese peroxidase (MnP - EC 1.11.1.13) and lignin peroxidase (LiP - EC 1.11.1.14) secreted by WRF, synergistically depolymerize and mineralize lignin under aerobic conditions (Sharma *et al.,* 2017). This enzymatic potential makes ligninolytic fungi highly valuable for various biotechnological applications, such as bioethanol and bioplastic production, bioremediation, delignification in industrial processes etc.

The current investigation focuses on the isolation and subsequent morphological and molecular identification of fungal strains possessing the potential to produce ligninolytic enzymes sourced specifically from the diverse ecological niches of the South Gujarat region. This approach aims to characterize novel microbial candidates capable of efficient lignin degradation for biotechnological applications.

Material and Methods

Materials Used:

Potato Dextrose Agar (Qualigens), Guaiacol (Hi-media), Hydrogen Peroxide (ICL), Thiamine HCL, NH_4NO_3, $CuSO_4.5H_2O$, $CoCl_2.6H_2O$, $MnSO_4.H_2O$, ammonium ferric citrate, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, $ZnSO_4.7H_2O$, KH_2PO_4 (SD fine chemicals, Mumbai, India), DNA Extraction Kit [SLS Lifesciences Pvt. Ltd.]. All other chemicals used were of analytical grade.Wheat straw collected locally and used as lignocellulosic substrate.

Sample Collection:

Different forms of samples like rotten wood, compost, municipal waste, soil, fruiting bodies were collected in clean plastic ziplock bags or clean plastic containers from various areas of South Gujarat (Chang *et al*., 2012; Sharma *et al*., 2017).

Isolation and Screening of Lignin Degrading Fungi

Among all samples, soil samples were serially diluted using sterile distilled water and then 0.1 ml sample spreaded onto sterile Potato dextrose agar plates. In case of rotten wood and fruiting bodies were cut into thin small portions and inoculated on potato dextrose agar (PDA)plates. Plates were incubated at 28 ± 3 °C for 5-10 days. Microscopic examination of all isolates were done through mounting with picric acid. The purified isolated fungi were transferred on PDA slant and stored at low temperature in refrigerators and were transferred at 20 days interval for maintenance.

(A) Primary - Qualitative screening for lignin degrading fungi:

For qualitative screening of isolates to check their ligninolytic potential, Czapek dox agar plates with appropriate substrates were used (Sharma *et al*., 2017).

i) Guaiacol plate assay:

Isolates were inoculated onto sterile Czapek dox agar plates supplemented with 2-3 drops of Guaiacol and incubated at room temperature for 7-12 days. Positive result was indicated by the formation of a brown zone around the colonies and indicating the production of laccase enzyme which oxidized guaiacol.

ii) Methylene blue dye decolorization plate assay:

Isolates were inoculated onto sterile Czapek dox agar plates supplemented with 0.5 ml 1% Methylene blue dye and incubated at 28 ± 3 °C for 7-12 days. Positive reaction is indicated by the formation of a clear zone around the colonies. Positive results indicate the production of lignin peroxidase enzyme which decolorize the polymeric dyes.

iii) Methyl orange dye decolorization plate assay:

Isolates were inoculated onto Czapek dox agar plates supplemented with 0.5 ml 1% Methyl orange dye and incubated at 28 ± 3 °C for 7 to 12 days. Positive reaction was indicated by the formation of a clear zone or decolorization around the colony. Positive results indicate the production of manganese peroxidase enzyme which decolorize the polymeric dye.

(B) Secondary - Quantitative screening for Laccase, Lignin peroxidase and Manganese peroxidase enzymes producing fungi:

For quantitative screening of selected isolates, flasks containing 5.0 g wheat straw as lignocellulosic substrate moistened with Asther's medium were inoculated with individual isolates aseptically and incubated for different time courses at 28 ± 3 °C under solid state fermentation.

i) Determination of laccase activity:

Filtrate from flasks at different intervals (Day - 3, 6, 9, 12 and 15) were collected and activity was checked using a method described by Coll *et al*., (1993) with slight modifications. Reactive mixture was prepared by adding 0.5 ml 46 mM guaiacol, 2 ml sodium acetate buffer, 0.5 ml crude enzyme and 0.5 ml distilled water. Blank solution contained above reaction mixture but buffer was used instead of crude enzyme. Absorbance was read at 440 nm. The laccase enzyme activity was

calculated by using an extinction coefficient of 0.6740 $M^{-1}.cm^{-1}$. One unit is defined as the amount of enzyme necessary to liberate 1 µM of substrate per minute or ketals.

$$\text{Enzyme Activity } (U/ml) = \frac{\text{Absorbance at 440 nm} \times \text{Total volume of reaction mixture (ml)}}{\text{Incubation time (min)} \times \text{Extinction Coefficient } (M^{-1}\, cm^{-1}) \times \text{enzyme Volume (ml)}}$$

ii) Determination of lignin peroxidase activity:

Filtrates from flasks at different intervals (Day - 3, 6, 9, 12 and 15) were collected and activity was checked according to Bergmeyer, 1974; Sarkanen *et al*., (1991). Reactive mixture was prepared by adding 0.5 ml of 46mM guaiacol solution, 2 ml of sodium acetate buffer (50mM, pH 4.6), 0.5 ml of crude enzyme and 0.5 ml of substrate solution of H_2O_2 (12.3 mM). Blank solution contained the above reaction mixture but crude enzyme was replaced by buffer. Absorbance was read at 440 nm. The lignin peroxidase enzyme activity was calculated using an extinction coefficient of 0.6740 $M^{-1}.cm^{-1}$ and activity expressed as one unit, defined as the amount of enzyme necessary to oxidize 1 µM of substrate (H_2O_2) per minute or ketals (Banakar *et al.,* 2014).

$$\text{Enzyme Activity } (U/ml) = \frac{\text{Absorbance at 440 nm} \times \text{Total volume of reaction mixture (ml)}}{\text{Incubation time (min)} \times \text{Extinction Coefficient } (M^{-1}\, cm^{-1}) \times \text{enzyme Volume (ml)}}$$

iii) Determination of manganese peroxidase activity:

Filtrates from flasks at different intervals (Day - 3, 6, 9, 12 and 15) were collected and activity was checked according to Kheti *et al.,* (2023). Reaction mixture prepared by adding 1 ml sodium succinate buffer pH 4.5 (50 mM), 1 ml sodium lactate pH 5.0 (50 mM), 0.4 ml 0.1 mM manganese sulphate, 0.7 ml 0.1 mM phenol red, 0.4 ml 50 mM H2O2 and 0.5 ml of crude enzyme. The absorbance of one millilitre of the reaction mixture in 40 µl of 5 N NaOH was measured at 610 nm.

Molecular Identification of Fungal Isolates Through ITS Region

DNA was isolated from purified fungal isolates by using a DNA isolation kit from Saffron Lifesciences Pvt. Ltd. DNA sequencing reaction was carried out with ITS1 & ITS4 primers using BDT v3.1 Cycle Sequencing Kit on ABI 3500xl Genetic Analyzer at Genexplore diagnostic & research center Pvt. Ltd.

Result and Discussion

Sample Collection, Isolation and Screening

A total of 15 different samples were collected, from which 18 different fungi were isolated. All collected fungal isolates were checked for their ability to produce laccase, lignin peroxidase and manganese peroxidase enzymes using plate method which had guaiacol, methylene blue and methyl orange as their respective substrates. Brown halo around growth, decolorization of methylene blue and methyl orange indicates ability to produce laccase, lignin peroxidase and manganese peroxidase enzyme production respectively. Results of primary screening are mentioned in Table 1.

Quantitative detection (secondary screening) of ligninolytic enzyme production was carried out using wheat straw as a solid substrate and Asther's medium as a moistening agent under solid state fermentation. Among eighteen, four isolates were subjected to secondary screening through enzyme assays. Three fungal isolates labelled F5, F8 and F9 were found to have good potential for production of Lac and LiP enzymes while no fungal isolate was able to produce a good amount of MnP enzyme during secondary screening. F5 showed Lac and LiP activity of 261.32 U/g and 503.1 U/g respectively, whereas F8 and F9 showed 434.29 U/g and 207.67 U/g laccase activity and 430.5 U/g and 330.6 U/g P activity, respectively (Figure - 2 a,b). Study conducted by Sharma *et al*., (2017) reported highest Lac activity 17.85±.92 g/uds while highest MnP and LiP activities were found to be 1.34±.025 g/uds and 1.78±.041 g/uds respectively by different fungal strains isolated from rotten wood and agricultural soil.

Table 1: Sampling and screening of fungal isolates for their ligninolytic potential

Name of Isolate	Type of sample collected and location	Name of Indicator		
		Guaiacol	Methylene Blue	Methyl Orange
F1	Soil from an organic farm, Desad-Gandevi.	+	+	+
F2A	Rotten wood, Dang forest-Ahwa.	-	-	-
F2B		-	-	-
F3	Municipal waste, Auranga river dump site-Valsad.	-	-	
F4	Soil from garden, Abrama- Valsad.	-	-	-
F5	Fruiting body, Dang forest-Ahwa.	+	+	+
F6	Soil (wet) from near lake area, Chikhli-Navsari.	-	-	-
F7	Soil from a farm, Tapawad-Valsad.	-	-	-
F8	Fruiting body, Ozar - Dharmpur.	+	+	+
F9	Rotten wood, Lakhampore-Pardi.	+	+	+
F10	Compost, Parnera- Valsad.	-	-	-
F11	Soil from kitchen garden, Atul colony-Valsad.	-	-	-
F12	Fruiting body, Tithal road-Valsad.	-	-	-
F13A	Soil from rice paddy field, Hanmatmal-Dharampur.	-	-	-
F13B		-	-	-
F14A	Cow dung, Tapawad-Valsad.	-	-	-
F14B		-	-	-
F15	Soil from expired pesticide dumpling area, Vapi GIDC.	-	-	-

The fungal isolates were grown on their respective agar plates containing indicator at 28±4ºC
+ = Positive for ligninolytic potential
- = Negative for ligninolytic potential

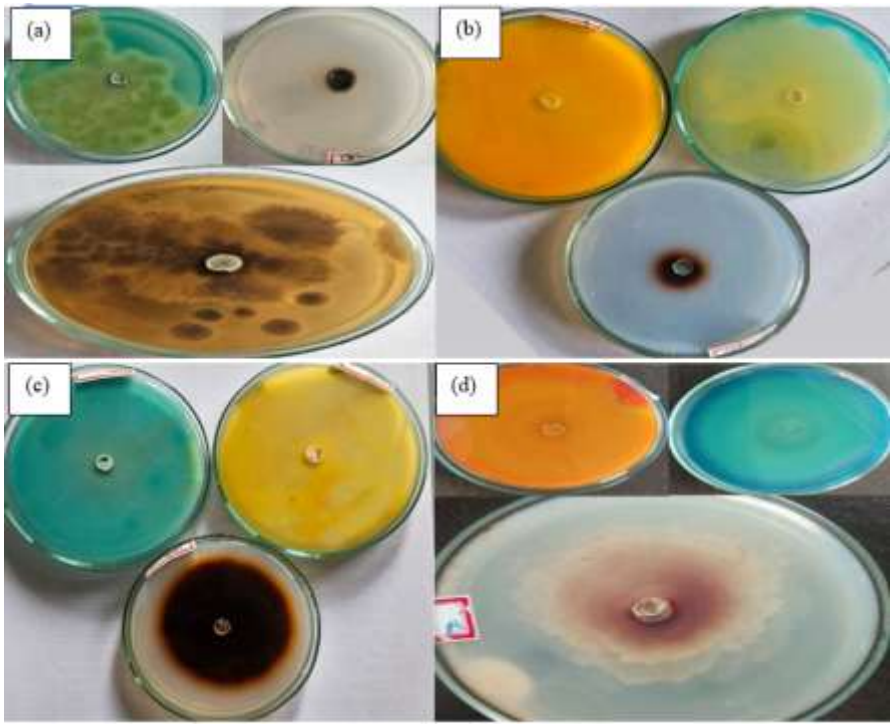

Fig. 1: Qualitative screening of ligninolytic fungi (a) F1 (b) F5 (c) F8 (d) F9

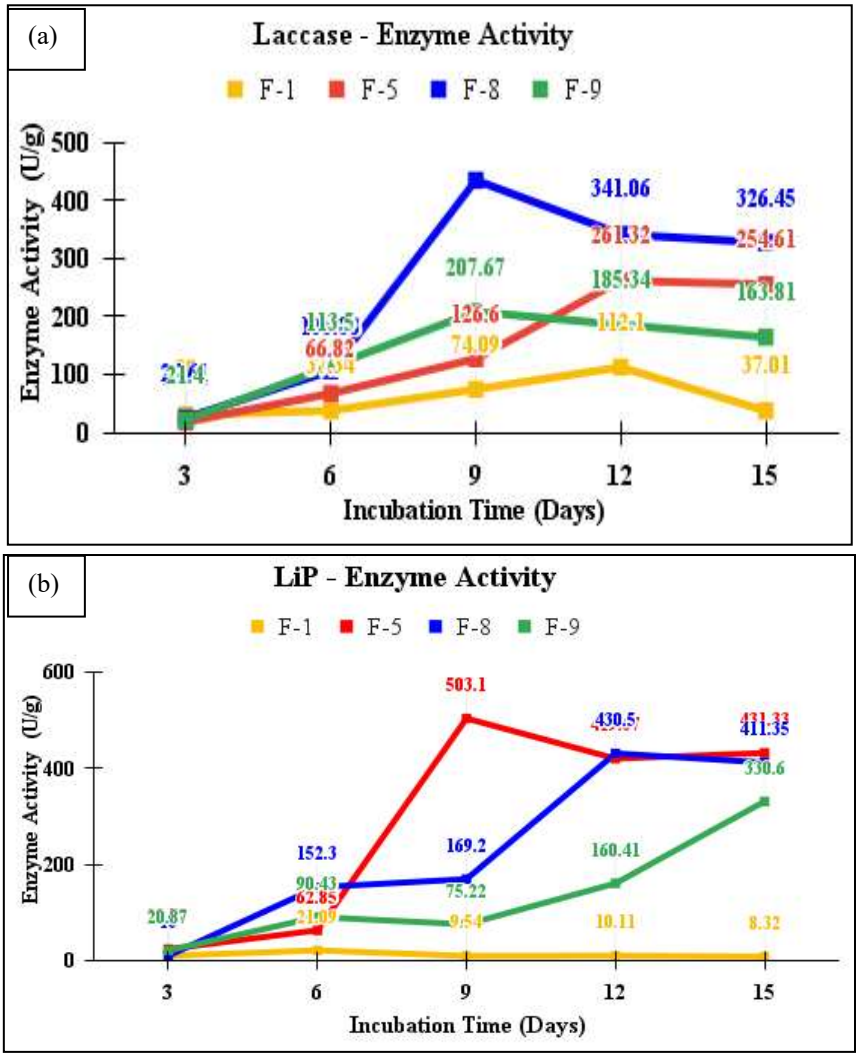

Fig. 2: Secondary screening through quantitative analysis (a) result of Laccase enzyme activity (b) result of LiP enzyme activity

Identification of Fungal Isolates

Morphological examination of F5 shows that the strain flourished, covering the entire petri-plate surface in 5-7 days with cottony texture while F9 was found to be a slow grower which took 9-11 days showing concentric rings pattern. Isolate designated as F8 showed chocky white texture and its growth covered the surface of the entire petri-plate within 6-8 days (Fig. 3).

Microscopic examination of all three isolates is shown in Fig. 4. In the microscopic field of F5 fugal mounting, septate hyphae with spindle shaped microconidia as well as sickle shaped macroconidia were observed. Cylindrical shaped basidiospores and paraphyses were found present in F8 while conidiophores were found erect and then branched laterally in F9 fungal isolate.

The identification of fungal cultures was further confirmed by advance studies on their ITS I and ITS 2 gene sequences carried out by Genexplore diagnostic & research center Pvt. Ltd. Based on nucleotide homology analysis & phylogenetic analysis focusing ITS 1 and ITS 2 gene sequences of isolates revealed that isolate designated F5 is *Fusarium chlamydosporum,* F8 is *Polyporus benetostus* and F9 is *Trichoderma erinaceum*. Figure -5 (a) having aligned sequence data of 569 bp of *Fusarium chlamydosporum*, (b) 651 bp of *Polyporus benetostus and* (c) 519 bp of *Trichoderma erinaceum* were submitted to National Centre for Biotechnology Information (NCBI) GenBank and assigned an accession no. PX482664, PQ348730 and PQ349085 respectively. The boot strapped unrooted trees were constructed by the neighbor-joining (NJ) method through MEGA software for fungal isolates F5, F8 and F9 from the distance data generated by multiple alignment of the nucleotide sequences shown in Fig. 6, 7 and 8 respectively.

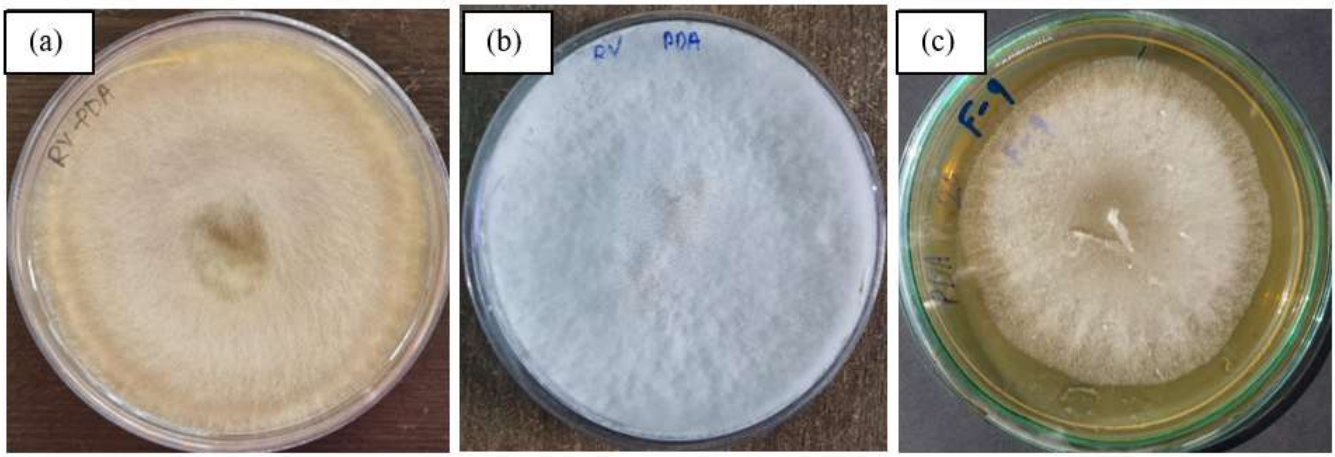

Fig. 3: Morphological characteristics of fungal isolates (a) F5 (b) F8 (c) F9

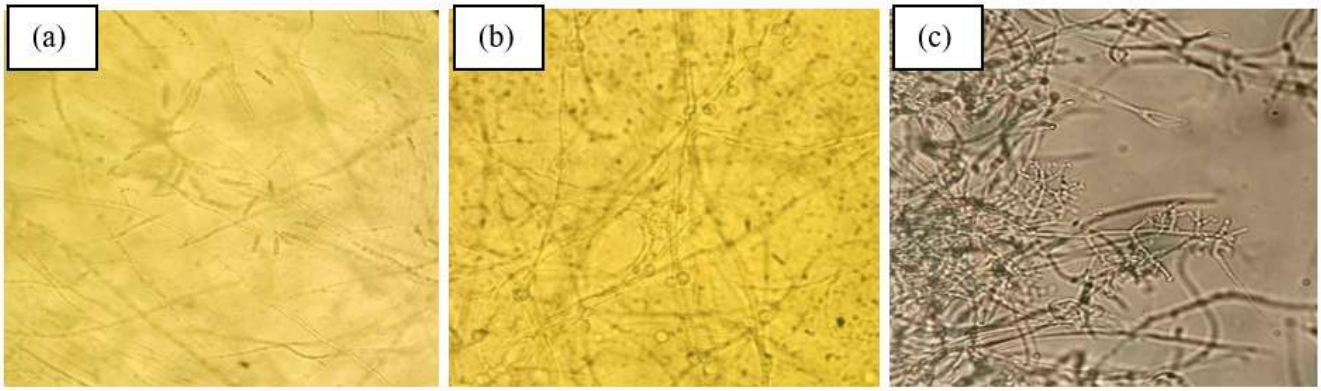

Fig. 4: Microscopic observation of fungal isolates (a) F5 (b) F8 (c) F9 mounted in picric acid

ATCCCTTCGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGA
ACATACCTATACGTTGCCTCGGCGGATCAGCCCGCGCCCCGTAAAACGGGACGGCCCGCCCGAG
GACCCTAAACTCTGTTTTTAGTGGAACTTCTGAGTAAAACAAACAAATAAATCAAAACTTTCAA
CAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCA
TGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTGTTGGGACTCGCGGTAACCCGCG
TTCCCCAAATCGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAATCATACACCTCGTTACTG
GTAATCGTCGCGGCCACGCCGTAAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAA
TACCCGCTGAACTTAAGCATATCAATAAGCGGAGG

Fig. 5: (a) Aligned sequence data F5 length (569 bp)

CCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAGGTCAGATTGTCAAAAG
TTGTCCGAAGACGATTAGAAGCATAGACATTTTGTTATACCGCACTACTAACAGTGTAGACAAT
TATCACACTGGAGAGTAGCAGTATTATCTCTGCTAATGCATTTAAGAGGAGCTGGTAATACAAT
CATTACCTGCAAAAACCTCCAAGTCCAACACACCCTTATCCGCAAAGATTTGGGGTATTGAGAA
TACCATGACACTCAAACAGGCATGCCCTTCGGAATACCAAAGGGCGCAAGATGCGTTCAAAATT
CGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCG
AGAGCCAAGAGATCCGTTGCTGAAAGTTGTATTTATTGCGTTATACGCGGATGACATTCTAAAA
CTGAAGCGTTTGTAAAAGCATAGGTCAGTATTTCCAAAGTCAATAAAGACCTTTTCTATACCAA
ACCTATGTAAAGTGCACAGAGGTGTATGAAATGGAATGAACAGAATGTGCACATGCCCGGTTGG
GGCCAGCTACAATTCCGCCATAAATTCATTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTT
GTTAC

Fig. 5: (b) Aligned sequence data F8 length (651 bp)

GTTGGGTGTTTTACGGACGTGGACGCGCCGCGCTCCCGGTGCGAGTTGTGCAACTACTGCGCAG
GAGAGGCTGCGGCGAGACCGCCACTGTATTTCGGGGCCGGGATCCCGTCTGAGGGGTTCCCGAT
CCCCAACGCCGACCCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCC
AGAATACTGGCGGGCGCAAGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACAT
TACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTT
ATTCATTTTGAATTTTTTGCTCAGAGCTGTAAGAAATAACGTCCGCGAGGGACTAAGTAAAGAG
TTTGGTTGGTCCCTCCGGCGGGCGCCTGGTTCCGGGGCTGCGACCACCCGGGGCGTGACCCCGC
CGAGGCAACAGTTTGGTATGGTTCACATTGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCT

Fig. 5: (c) Aligned sequence data F9 length (519 bp)

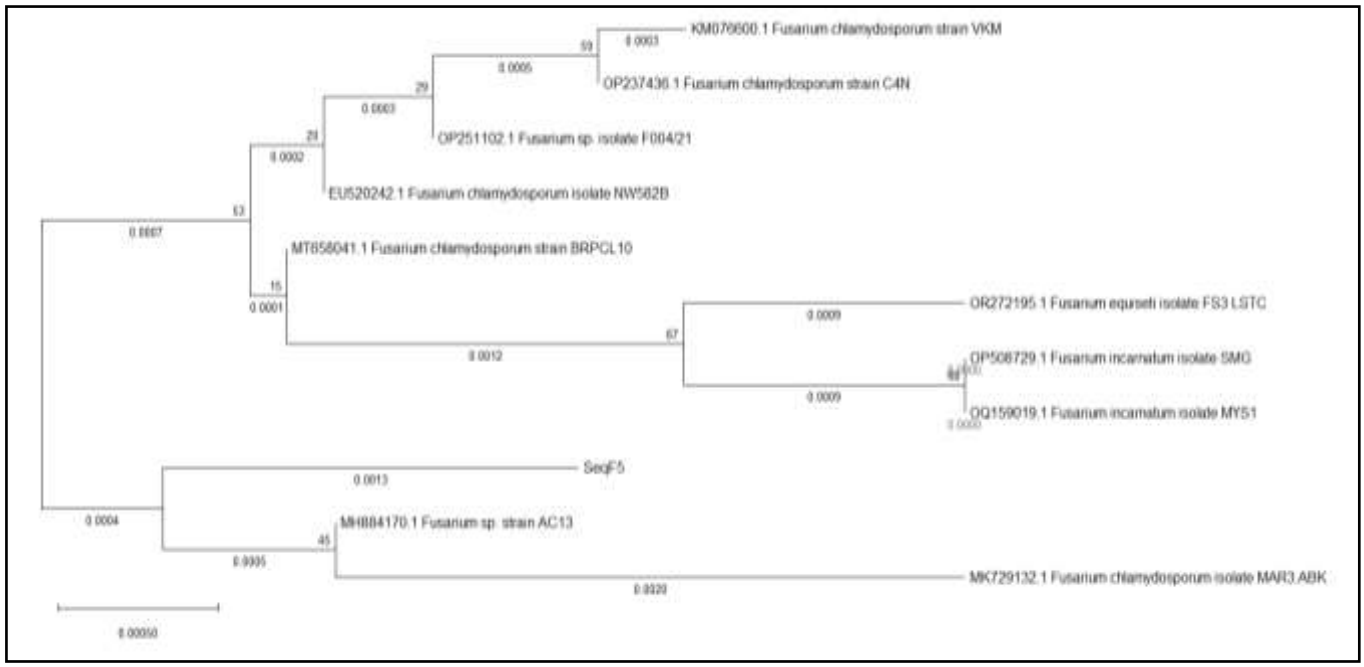

Fig. 6: The bootstrapped unrooted tree of F5 constructed by NJ method

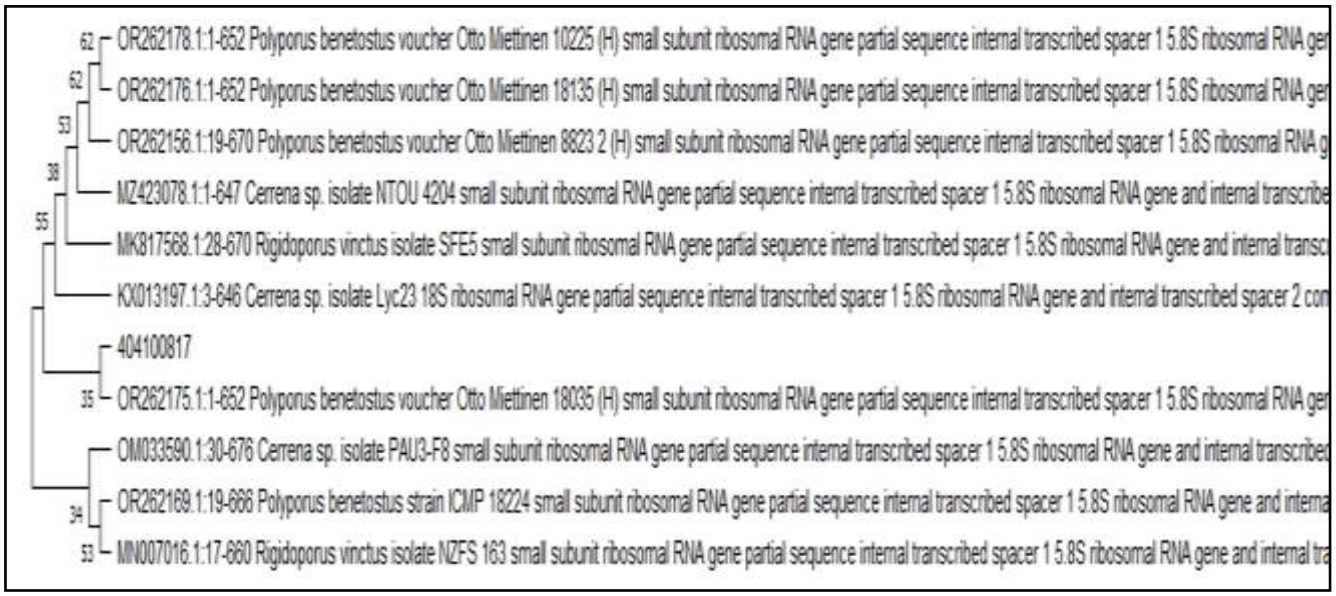

Fig. 7: The bootstrapped unrooted tree of F8 constructed by NJ method

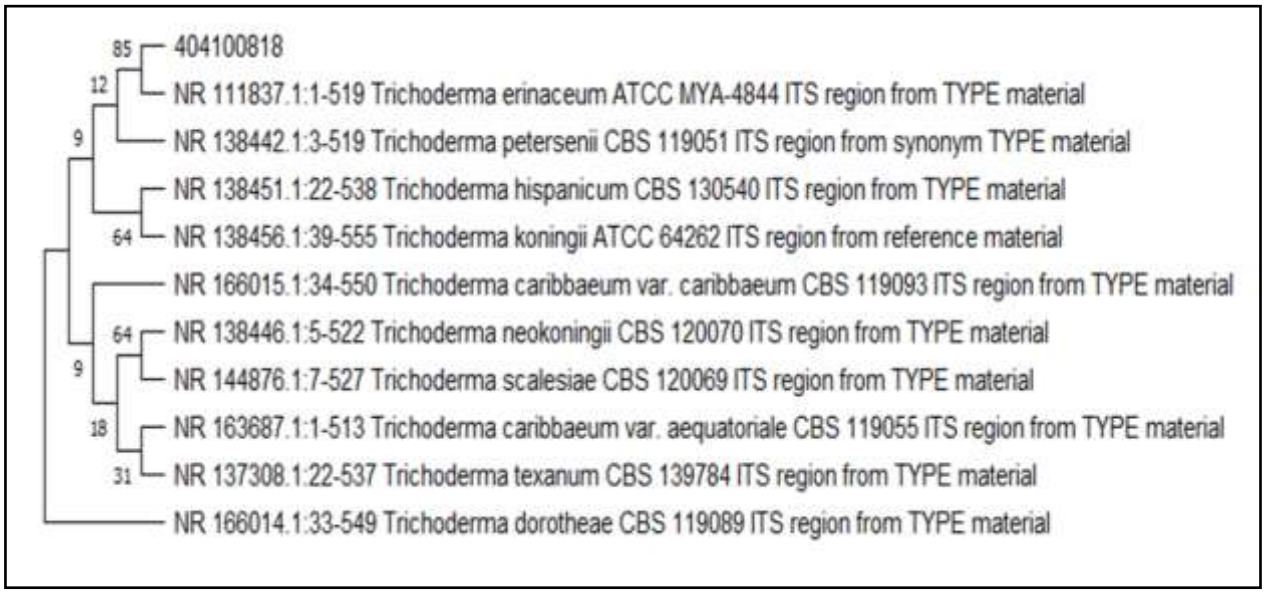

Fig. 8: The bootstrapped unrooted tree of F9 constructed by NJ method

Conclusion

The present study was undertaken with the aim of isolation and screening of ligninolytic fungi from the South Gujarat region. Three fungal strains displayed all three extracellular ligninolytic enzymes (Lac, MnP and LiP) activities. Highest Lac activity was produced by F8 (434.29 U/g) while highest LiP activities were produced by F9 (503.1 U/g). This preliminary study requires further research focusing on compatibility testing for co-culturing and optimization of cultural and environmental parameters to enhance enzyme production. These isolates seem promising candidates for both enzyme production (Lac and LiP) and can be useful in various applications including dye decolorization, bioconversion of lignocellulosic biomass into ethanol, degradation of pesticides etc.

Authors' Contributions

R.V and Dr. M.D both designed the study, R.V performed the experiments, collected, conducted data analysis and drafted the manuscript. Dr. M.D reviewed and edited the manuscript. Both authors approved the final form of the manuscript.

Conflict of Interest

The authors declare no conflict of interest with present research.

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References

Abd El Monssef RA, Hassan EA & Ramadan EM (2016) Production of laccase enzyme for their potential

application to decolorize fungal pigments on aging paper and parchment. *Annals of Agricultural Sciences* **61**(1): 145-154. DOI: 10.1016/j.aoas.2015.11.007

Banakar SP & Thippeswamy B (2014) Isolation and partial purification of fungal ligninolytic enzymes from the forest soil fungi isolated from Bhadra Wildlife Sanctuary. *Frontiers in Biology* **9**(4): 291-299. DOI: 10.1007/s11515-014-1319-x

Bergmeyer HU (1974). Methods of Enzymatic Analysis 1, 2nd Ed, New York: Academic Press, 1–495

Bergmeyer H U (Ed.) (2012) *Methods of enzymatic analysis*. Elsevier. Aebi, H. (1974). Catalase. In Methods of enzymatic analysis (pp. 673-684). Academic press. DOI: 10.1016/B978-0-12-091302-2.50032-3

Brown ME & Chang MC (2014) Exploring bacterial lignin degradation. *Current opinion in chemical biology* **19**: 1-7. DOI: 10.1016/j.cbpa.2013.11.015

Bugg TD, Ahmad M, Hardiman EM & Singh R (2011) The emerging role for bacteria in lignin degradation and bio-product formation. *Current opinion in biotechnology* **22**(3): 394-400. DOI: 10.1016/j.copbio.2010.10.009

Chang AJ, Fan J & Wen X (2012) Screening of fungi capable of highly selective degradation of lignin in rice straw. *International Biodeterioration & Biodegradation* **72**: 26-30. DOI: 10.1016/j.ibiod.2012.04.013

Coll PM, Fernandez-Abalos JM, Villanueva JR, Santamaria R & Perez P (1993) Purification and characterization of a phenoloxidase (laccase) from the lignin-degrading basidiomycete PM1 (CECT 2971). *Applied and environmental microbiology* **59**(8): 2607-2613. DOI: 10.1128/aem.59.8.2607-2613.1993

De Gonzalo G, Colpa DI, Habib MH & Fraaije MW (2016) Bacterial enzymes involved in lignin degradation. *Journal of biotechnology* **236**: 110-119. DOI: 10.1016/j.jbiotec.2016.08.011

Gilca IA, Ghitescu RE, Puitel AC & Popa VI (2014) Preparation of lignin nanoparticles by chemical modification. *Iranian Polymer Journal* **23**(5): 355-363. DOI: 10.1007/s13726-014-0232-0

Glenn JK & Gold MH (1983) Decolorization of several polymeric dyes by the lignin-degrading basidiomycete Phanerochaete chrysosporium. *Applied and Environmental Microbiology* **45**(6): 1741-1747. DOI: 10.1128/aem.45.6.1741-1747.1983

Huang XF, Santhanam N, Badri DV, Hunter WJ, Manter DK, Decker SR & Reardon KF (2013) Isolation and characterization of lignin-degrading bacteria from rainforest soils. *Biotechnology and bioengineering* **110**(6): 1616-1626. DOI: 10.1002/bit.24833

Kheti NK, Rath S & Thatoi H (2023) Screening and optimization of manganese peroxidase (MnP) production by Pseudoduganella violacea (SMB4), a bacterial isolate from Similipal Biosphere Reserve, Odisha and evaluation of Maillard reaction products degradation. *Sustainable Chemistry for the Environment* **2:** 100009. DOI: 10.1016/j.scenv.2023.100009

Kumar P, Barrett DM, Delwiche MJ & Stroeve P (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & engineering chemistry research* **48**(8): 3713-3729. DOI: 10.1021/ie801542g

Mathews SL, Grunden AM & Pawlak J (2016) Degradation of lignocellulose and lignin by Paenibacillus glucanolyticus. *International Biodeterioration & Biodegradation* **110**: 79-86. DOI: 10.1016/j.ibiod.2016.02.012

Ragauskas AJ, Beckham GT, Biddy MJ, Chandra R, Chen F, Davis MF & Wyman CE (2014) Lignin valorization: improving lignin processing in the biorefinery. *Science* **344**(6185): 1246843. DOI: 10.1126/science.1246843

Salvachúa D, Karp EM, Nimlos CT, Vardon DR & Beckham GT (2015) Towards lignin consolidated bioprocessing: simultaneous lignin depolymerization and product generation by bacteria. *Green Chemistry* **17**(11): 4951-4967. DOI: 10.1039/C5GC01165E

Sánchez C (2009) Lignocellulosic residues: biodegradation and bioconversion by fungi. *Biotechnology advances* **27**(2): 185-194. DOI: 10.1016/j.biotechadv.2008.11.001

Sarkanen S, Razal RA, Piccariello T, Yamamoto E, Lewis NG (1991) Lignin peroxidase: toward a clarification of its role *in vivo*. *J Biol Chem* **266**(6): 3636–3643. DOI: 10.1016/S0021-9258(19)67842-7

Sharma A, Aggarwal NK & Yadav A (2017) Isolation and screening of lignolytic fungi from various ecological niches. *Universal Journal of Microbiology Research* **5**(2): 25-34. DOI: 10.13189/ujmr.2017.050202

Wang Y, Liu Q, Yan L, Gao Y, Wang Y & Wang W (2013) A novel lignin degradation bacterial consortium for efficient pulping. *Bioresource Technology* **139:** 113-119. DOI: 10.1016/j.biortech.2013.04.033

Yang J, Yuan H, Wang H & Chen W (2005). Purification and characterization of lignin peroxidases from Penicillium decumbens P6. *World Journal of microbiology and Biotechnology* **21**(4): 435-440. DOI: 10.1007/s11274-004-1876-2

Yang S, Yuan TQ, Li MF & Sun RC (2015) Hydrothermal degradation of lignin: products analysis for phenol formaldehyde adhesive synthesis. *International journal of biological macromolecules* **72**: 54-62. DOI: 10.1016/j.ijbiomac.2014.07.048