Poll Res. 38 (3) : 236-240 (2019)
Copyright © EM International
ISSN 0257–8050

ISOLATION AND SCREENING OF NOVEL MICROBIAL STRAINS EXHIBITING LIGNOLYTIC POTENTIAL

K. SUDHARAMESHWARI* AND G. PAVYA

Department of PG Biochemistry, V.V.Vanniaperumal College for women, Virudhunagar, Tamilnadu, India

(Received 2 January, 2019; accepted 15 March, 2019)

ABSTRACT

In this study, microbes were isolated from old coir pith and characterized lignin degraders and its lignolytic potential is evaluated by decolourization of dye, enzyme activity and biomass production. The bacterial strains were isolated, based on the p- Anisidine degrading capacity and intensity of browning and decolourization. Among these strains only seven isolates shows positive results based on their lignolytic activity. Within seven isolates based on their morphology two isolates were identified as fungi. The decolourization of methylene blue has been used as an indicator of lignin peroxidase enzyme activity. Change in colour of the medium from blue to clear zone was observed for four isolates. The predominant methylene blue decolorizes were isolates 3,6,7,5. Among the isolate, isolate seven showed the highest degrading activity which were selected for further identification by partial sequence analysis of their 18S rRNA genes. The isolate seven is identified as *Aspergillus flavus*. The sequence is deposited in NCBI GenBank with the accession number MH161388.

KEY WORDS : Lignin degrader, Isolation and identification of microbes by sequencing

INTRODUCTION

Lignin is the most structurally advanced macromolecule possessing a high relative molecular mass and therefore the most recalcitrant, consisting of assorted biologically stable linkages (Perez *et al.,* 2001). The lignocelluloses material of plant consists of polysaccharide, hemicelluloses, and lignin. The polymer degrading microorganism strains were known by that has the flexibility to provide lignolytic catalyst to decolorize the stain dyes (Mukesh Chander and Inderdeep 2015). The chemicals that employed in business as dyes area unit nitro, nitroso, vat, sulphur, xanthenes dyes etc. Such in depth use of color usually results in drawback of colored waste waters, which require pre-treatment for color removal before its disposal (Malaviya *et al.,* 2012). The waste water treatment system used area unit unable to finish take away the recalcitrant dyes from the effluents. The dyes area unit discharged into the aquatic and terrestrial surroundings through the effluents rising from the textile and color industries (Chander and Arora , 2014). These dyes should 1st take up by the microorganism cell for degradation by the extracellular enzymes (Coulibaly *et al.,* 2003). To solve this drawback during this study, microbes were isolated from recent fiber pith drop soil and its lignolytic potential is evaluated by decolourization of dye, catalyst activity and biomass production.

MATERIALS AND METHODS

Isolation of Micro organisms

Lignin degrading microorganism was isolated from coirpith dumped soil, Thambipatti, Tamilnadu, India. Both liquid as well as solid samples were collected in a sterilized bottle. Different kinds of microorganisms are present in the soil depending on the soil type, moisture, and other environmental factors. By a single method it is impossible to isolate all the microorganisms since such a great variety of organisms are present in the soil. Therefore it is necessary to use different types of media for the growth of microorganisms. Serial dilution technique

***Corresponding author's email:** sudhavvvc@gmail.com

and plating on glucose peptone agar medium, glycerol yeast extract agar medium and nutrient medium can be used to determine the number of variable microorganisms.

Screening of microorganisms

Lignolytic fungi or Actinomycetes was identified by Bavendamm test. Lignolytic bacteria or Actinomycetes was isolated by nutrient agar medium. The lignolytic enzymes laccase, catechol oxidase and lignin peroxidase enzymes were detected.

Lignolytic activity

The bacterial isolates were additional screened exploitation methylene blue dye as an indicator. The microbes possess lignolytic enzymes undergoes oxidization of indicator dye. The isolated bacteria were patterned on methylene blue indicator dye (0.25 g/L) containing LB agar plate. The plates were incubated at 30 °C for 72 h. The agar plates were monitored daily for microbial growth and decolourization of the methylene blue dyes (Sasikumar *et al*., 2014).

Effect of agro waste in laccase production

In 250 mL of round shape flask, 3g of coirpith (agro waste) was moistened by H2O and it is sterilised. Once autoclaving cooled it within bedded hood and a bit of YGA with recent culture took exploitation sterile sharp knife and inoculated within the round shape flask containing sterile agro wastes and incubated for 10 days in 28°C, once ten days sterilized metallic element acetate buffer a 100 mL (pH 5) further within that round shape flask and centrifuged. The supernatant was used as crude extract containing laccase accelerator. Guaiacol used as substrate for accelerator activity determination exploitation UV- photometer at 450 nm.

Decolourization study of ordinarily used industrial dyes

All the positive isolates obtained were tested against some wide and ordinarily used Rhodamine dye. Within the experiment 5000 ppm concentration of business dyes were used for decolourization proportion determination in minimal salt medium. The entire decolourization % was determined from the 15th day treated sample exploitation this formula (Majolagbe *et al*., 2012; Rajendran *et al*., 2012).

$$\text{Decolourization proportion (\%)} = \frac{\text{(Abs. Initial)-(Abs. Final)}\times 100}{\text{(Abs. Initial)}}$$

Abs. initial=absorbance on first day; Abs. final=absorbance on fifteenth day

Alteration in Biomass production

After 15 days of incubation, biomass (mg/L) was measured. Throughout this study growth determined to indirectly correlate accelerator production.

Production of laccase and its assay

The pre-inoculum was prepared prior to enzyme production by taking a loop full of colony from the master plate and inoculating in nutrient broth. The inoculated broth was left in the shaker at 37 °C for 24 h. About 1mL of the pre-inoculum was added to 100 mL of enzyme production media at 120 rpm at 37 °C orbital shaker. Following incubation of the production medium for appropriate period, the contents in the flask were centrifuged at 8000 rpm for 5 min and the supernatant was collected which is an extracellular enzyme. Laccase assay was carried out using guaiacol method. The concentration of protein in crude sample was determined by Lowry *et al*. (1951).

Identification of best strain

After isolation of genomic DNA from the Isolate seven (best isolate), the genomic DNA was amplified exploitation universal primer ITS-1 and 4 (internal transcribe spacer) and run through I Chronicles agarose in presence of one kb marker. Amplified product refined exploitation image PCR shut down kit (Millipore). Send the refined PCR product for sequencing. Sequencing reactions were performed employing a ABI PRISM® BigDyeTM exterminator Cycle Sequencing Kits with AmpliTaq® DNA enzyme (FS enzyme) (Applied Biosystems). Single-pass sequencing was performed on every templet exploitation below 18s rRNA universal primers. The fluorescent-labeled fragments were refined from the unorganized terminators with an alcohol precipitation protocol. The samples were resuspended in H_2O and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). when sequencing, the strain identification was created by BLAST (basic local alignment search tool) and also the evolutionary relationship determined by the program MUSCLE three. 7 was used for multiple alignments of sequences (Edwards *et al*., 1989). The ensuing aligned sequences were corrected mistreatment the program Gblocks zero.91b. This

Gblocks eliminates poorly aligned positions and divergent regions (Talavera and Castresana, 2007). PhyML was shown to be as correct as alternative existing evolution programs exploitation simulated information, whereas being one order of magnitude quicker.

RESULTS AND DISCUSSION

In this study various aspects related to the lignin degradation was assessed.

Isolation of Lignin degrading microorganisms

Soil microorganisms are grouped into bacteria, fungi, algae and protozoa. Microorganisms are responsible for the numerous reactions that take place in the soil. Some of the reactions brought about by the microorganisms in the soil are highly beneficial; it becomes a suitable medium for plant and animal life (Lily, 1957). In our environment numerous microorganisms that have the degrading capacity and it convert them into organic manure in the soil. The current study was carried out by collecting the coir dumped soil and from that the different microorganisms were observed.

Screening of lignolytic microorganisms:

The strains were found to have a high potential of degrading lignin of tannic acid agar plate. The circular clearance zones were formed about around the culture. This method of identification was supported by Brajeshori Korjam *et al*., 2000. The bacterial strains were isolated, based on the p-Anisidine degrading capacity and intensity of browning and decolourization.

Among these strains only 7 isolates shows positive results based on their lignolytic activity. Within 7 isolates based on their morphology 2 isolates were identified as fungi. The lignolytic microbes showed clear zone of lignin degradation in each plate shown in Fig. 1.

Qualitative Detection of Lignolytic Enzymes

The extracellular production of these enzymes based on change in medium colour and visual detection has been proposed and evaluated by a simple petriplate screening procedure in solid medium. Nutrient agar medium plate of p- Anisidine degrading bacteria showed change in medium colour to purple by 1 hour which remained for upto 24 hours. Isolate 4 and 6 shows any colour change till 24 hours and this indicates absence of laccase enzyme in the medium. Similarly, all the seven isolates showed the presence of the enzymes catechol oxidase and lignin peroxidase which was detected in terms of change in colour of agar medium upon reaction with their specific substrates. The p- Anisidine degrading bacteria showed development of brown colour before 1 hour of initiating the reaction. Our results coincide with previous reports, Geetha and Sudharameshwari (2003) revealed *Pleurotus sp.* has maximum clearance and Amutha and Abhijit (2015) has observed *Trametesversicolor has* maximum clearance. White rot fungi are the most potent lignin degrader and have been surveyed for their capacity to produce these enzymes. There is a little information on the lignolytic enzyme of brown rot fungi, soft Aspergillus species. Screening of lignolytic microorganisms were done using MSM agar containing lignin added directly to the MSM agar medium as the sole carbon sources. The decolourization of methylene blue has been used as an indicator of lignin peroxidase enzyme activity (Ferreira-leitao *et al*., 2007). Change in colour of the medium from blue to clear zone was observed for 4 isolates. The predominant methylene blue decolourizers were isolates 3,6,7,5. The methylene blue decolourisation of seven isolates was shown in Fig. 2. Based on qualitative detection of lignolytic enzyme was tabulated in Table 1.

Isolate 3, 5 and isolate 7 were highly degrading capacity. After fifteen days of incubation, biomass (mg/L) was measured were Tabulated in Table two. Throughout this study growth was calculable to indirectly correlate protein production. The rise of microbial growth directly proportional to the laccase

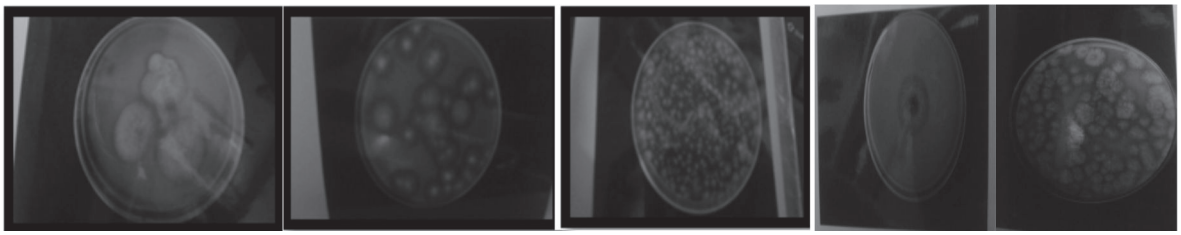

Fig. 1. Screening of lignolytic microorganisms

Table 1. Qualitative detection of lignolytic enzyme

Isolate	1	2	3	4	5	6	7
Laccase enzyme	+	+	+	-	+	-	+
Catechol oxidase	+	+	+	+	+	+	+
Lignin peroxidase	+	+	+	+	+	+	+
Methylene blue	-	-	+	-	+	+	+

+ = Present ; - = absent.

Table 2. Depicts the variability of different positive strains growth, decolourisation of Rhodamine dye, laccase enzyme activity

Isolate	1	2	3	4	5	6	7
Biomass of samples (mg/l)	81.43	98.23	79.04	178.2	65.03	88.45	197.42
Percentage of decolourisation of Rhodamine dye	68.3	73.4	76.2	85.7	79.1	72.5	88.3
Enzyme activity (µM/min/mg protein)	0.000054	0.00001	0.00017	0.00034	0.00011	0.000007	0.0004

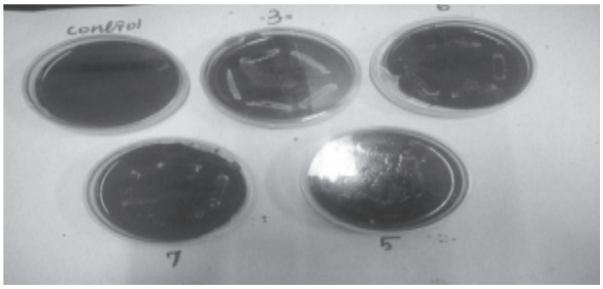

Fig. 2. Lignolytic enzyme activity in methylene blue

production (i.e.) here additional biomass means that additional quantity of laccase Protein production that is more practical in environmental facet. Biomass production increment of all positive isolates along with maximum biomass was seen in isolate7.

Isolate 5 has minimum biomass of 65.03mg/l. Our results supported with Amutha and Abhijit, (2015). During this experiment, the Rhodamine dye was used for degradation. Above 70% of decolourisation was seen after fifteenth day by all isolate expect isolate 1. Isolate 7 which are extremely higher decolorized than all positive strains. Our results coincides with Amutha and Abhijit, (2015) who revealed that PY-5 (*Trametesversicolor)* has maximum decolourization isolate 7. Effect of agro waste (coir pith) in laccase production was tabulated in table 4**.** Isolate 7 shown greater enzyme activity when compare to other isolates. Enzyme activity of isolate 4 is closely near to isolate 7 in agro waste. Our results coincide with Park *et al.,* 2006 and Amutha and Abhijit (2015) who reported that PY-5 has high laccase activity in the paddy straw.

This study shows among all positive isolates, solely the most effective result showing strain sequencing has done.

Identification

Sequence of ITS-1&ITS-4 compared with database and it showed the 100% sequence similarity with *Aspergillus flav*us. So the isolate 7 isolated from Thambipatti, India shown best environment friendly activity with respect to other positive isolates.

The dendrogram Figure 3 reveals the evolutionary relation of the best strain *Aspergillus flav*us of this study. The sequence is deposited in NCBI GenBank with the accession number MH161388.

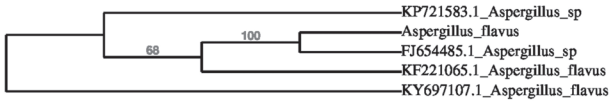

Fig. 3. Dendrogram of isolate 7 (*Aspergillus flavus*) constructed based on 18S rRNA sequences.

CONCLUSION

In this study, isolate the lignin degrading microorganisms from old coir pith dumped soil. Among the isolate, isolate 7 showed the highest degrading activity. The 18S rRNA gene of isolate 7 shared 100% similarity with that of *Aspergillus flavus* which has significant potential use in the applications for the treatment of lignin degradation and lignin related environment pollutants.

ACKNOWLEDGEMENT

The authors are grateful to the Management of V. V. Vanniaperumal College for Women, Virudhunagar,

Tamil Nadu, India for providing necessary facilities to carry out this research work. We are thankful to Yaazh Xenomics, Coimbatore for their valuable help in sequencing.

REFERENCES

Amutha C, Subramanian P, Manna A. 2004. Bioremediation its basic modern methods application a review. *Int. J. Trds. Appl. Microbio. Biotech*. 3 : 49-76.

Amutha, C. and Abhijit, M. 2015. Screening and isolation of laccase producers, Determination of optimal condition for growth, laccase production and choose the best strain. *Bioremediation and Biodegradation.* 6 : 4.

Brajeshori Korjam, Nileshc. Shaena and Sanjay Gupta.2000. Production and characterization of fungal cellulose from grow celluloisc waster. *Asian Jr. Microbiol. Biotech and Env. Sc.* 4 (3-4) : 113-120.

Chander, M. and Arora, D.S. 2014. Biodegradation of a dye by different white fungi on a novel agro residue based medium. *Lignocellulose*. 3 (1) : 37-50.

Coulibaly, L., Gourene, G. and Agathos, N.S. 2003. Utilisation of fungai for biotreatment of raw waste waters. *Afr. J. Biotechnol.* 2 : 620-630.

Edwards, U., Rogall, T., Blocker, H., Emde, M. and Bottger, E.C. 1989 Isolation and directcomplete nucleotide determination of entire genes: characterization of a gene coding for16S ribosomal RNA. *Nucleic Acids Res*. 17 : 7843-7853.

Ferreira-leitao, V.S., Andrade de Carvalho, M.E. and Bon, E.P.S. 2006. Lignin peroxidase efficiency for methylene blue decoloration: Comparison to reported methods. *Dye Pigment*. 4 : 230-236.

Geetha, M. and Sudharameshwari, K. 2003. Isolation of lignin degraders from Coir waste and evaluation of decomposition of coirpith with different microbes and their effect on growth of *Abelmoschus eseulentus.* M.Sc. Thesis, V.V.V College for women, Tamilnadu, India.

Lily, V.G. 1957. Soil microorganisms and their significance. *Coconut Bulletin*. 9 (3) : 286-289.

Lowry, OH, Rose Brough NJAL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *Journal Biol. Chem.* 193 : 265-275.

Majolagbe, O., Oloke, J., Deka-Boruah, H., Adetunji, C. and Borah, M. 2012. Extraction and purification of extracellular laccase from wild, mutants and hybrid strains of two white rot fungus & its applications in decolorization and liginolysis. *J. Microbio. Biotech. Food Sci*. 2 : 998-1016.

Malaviya, P., Rajesh, H. and Sharma, N. 2012. Impact of dyeing industry waste on germination and growth of pea. *J. Environ. Biol*. 33 : 1073-1075.

Mukesh Chander and Inderdeep Kaur, 2015. Bioprocess Laboratory, Department of Biotechnology, Khalsa College, Amritsar, Punjab, India. An industrial Dye Decolourization By Phlebiasp. *Int. J. Cur. Micro. Appl. Sci*. 4 (5) : 217-226.

Park, C., Lee, M., Lee, B., Kim, S.W., Chase, H.A., Lee, J. and Kim, S. 2006. Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii. Bioche. Eng*. J. 10 : 106-110.

Perez, J., Rubia, T.D.L., Martinez, J. and Kapley, A. 2001. Biodegradation and biological treatment of cellulose, hemicellulose and lignin: An overview. *Int. Microbiol.* 5 : 53-63.

Rajendran, R., Karthik, S.S., Yasodha, K. and Umamaheswari, K. 2012. Comparision of Fungal Laccase production on different solid substrates, Immobilized and its decolorization potential on synthetic textile dyes. *Institute of Integrative Omics and Applied Biotechnology.* 3 (5) :1-6.

Sasikumar, V., Priya. V., Shiv Shankar, D. and Sathish, Sekar, D. 2014. Isolation and Preliminary Screening of Lignin Degrading Microbes. *Journal of Academia and Industrial Research.* 3 (6) : 291-294.

Talavera, G. and Castresana, J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*. 56 :564-577.