

Original Research Article

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Isolation and Screening of Lignocellulose Degrading Fungi from Degraded Fruit Litter

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ABSTRACT

Keywords

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The objective of the study conducted was to screen & isolate fungi capable of degrading the fruit litter of *Albizia lebbbeck*. These fruit Biomass are often disposed by burning, which pose an environmental pollution problem. The fruit biomass can be potentially converted to various value added products like bio fuels, cheap energy sources for fermentation, improved animal feeds. Considering these facts the present work is focused on isolation of fruit litter degrading fungi. The fruit litter was allowed to degrade in specially made pits and soil sample from the degraded fruit was used for the isolation of fungi. Isolation was done by serial dilution method and direct inoculation method on czapekdox agar medium. 14 different fungi were isolated and they were tested qualitatively for their capability to release cellulases, xylanases and ligninases. Out of the 14 fungi isolated, Isolate 2(IS2) identified as *Aspergillus niger* had shown the potential of releasing of cellulases, xylanases and ligninases.

Introduction

Biomass is the mass of organic material from any biological material. A wide variety of biomass resources are available on earth for conversion in to bio products, which include whole plants, plant parts, plant constituents, processing by products, materials of marine origin and animal by-products, municipal and industrial wastes. These resources can be used to create new biomaterials (Howard *et al.*, 2003), Mehdi Dashtban, *et al.*, 2010). Lignocelluloses comprise the major structural component of woody plants and non woody plants such as grass. Lignocellulosic wastes are generated in large quantities by forestry and agricultural practices, paper and pulp

industries, agro industries, timber industries which pose an environmental pollution problem. Such wastes are also present in municipal solid wastes and animal wastes (Howard *et al.*, 2003; Mehdi Dashtban *et al.*, 2010). Much of the lignocellulose waste is often disposed of by biomass burning, which is considered a global phenomenon. However, the huge amounts of residual plant biomass considered as waste can potentially be converted in to various value added products including biofuels, chemicals, cheap chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients (Howard *et al.*, 2003).

Lignocellulolytic enzymes also have significant potential applications in various industries including chemicals, fuel, food, brewery and wine, animal and feed, textile and laundry, pulp and paper and agriculture (Mehdi dashtban *et al.*, 2009).

There are a number of studies with regard to wood and leaf litter decomposition, enzymes involved in the degradation process and recycling of nutrients, from different parts of the world. There are meagre studies with regard to fruit litter decomposition of *albizia lebbeck* and the enzymes involved in degradation process. The present investigation is undertaken to isolate fungi able to decompose fruit litter and the enzymes involved in the degradation of fruit litter of *albizia lebbeck*.

Materials and Methods

Fruit material

The fruit material was collected from Manakondur village, District Karimnagar, washed and shade dried.

Isolation of lignocellulose degrading fungi

The fruit litter was allowed to degrade in specially made pits. After a period of 3 months soil sample from the degraded fruit was collected and fungi were isolated by dilution plating technique on Czapekdox agar medium incorporated with chloramphenicol. The plates were incubated at RT 28 +/-2⁰C.

The plates were observed for growth daily and sub cultured on to fresh plates until pure fungal cultures were obtained. The pure cultures were maintained on slants by subculturing every four weeks. The isolated fungi were observed for their macroscopic and microscopic characteristics (Joseph C. Gilman).

Qualitative screening of the isolates for release of lignocellulolytic enzymes

Composition of basal medium (BSM) used

C₄H₁₂N₂O₆ – 5g/l, KH₂PO₄ – 1g/l, MgSO₄.7H₂O -0.5g/ml, Yeast extract – 0.1g/l, CaCl₂.2H₂O – 0.001g/l

Test for cellulase and xylanase activity (Stephen B. Pointing, 1999)

BSM incorporated with 2% carboxy methyl cellulose was used to grow the fungal isolates. The plates were stained with 1% Congo red solution followed by neutralization with 1M sodium chloride solution. Formation of clear zones around the colonies indicates the production of cellulase. BSM incorporated with 1% Xylan was used for the production of xylanases. The plates were flooded with iodine stain containing 1% iodine crystals, 2% KI. Based on the clearing zone xylanase producing organisms were screened. Xylan degradation around the colonies appears as yellow opaque area against a blue/reddish purple colour for undegraded xylan.

Test for Lignin degrading enzymes: (Stephen B. Pointing, 1999)

Azure B agar clearance

Lignin basal medium (LBM) was supplemented with 0.01% w/v Azure B and 1.6% w/v agar. 20% w/v Glucose was sterilized separately and added aseptically. The fungi was inoculated and incubated at 25⁰C in darkness. Clearance of blue coloured medium indicates production of LiP.

Lignin agar

LBM was supplemented with 0.25% w/v Lignin, inoculated with the fungus and incubated at 25⁰C. After 5 -10 days the plates

were flood with 1% w/v aqueous solution of $FeCl_3$ and $K_3[Fe(CN)_6]$ prepared freshly before use. Phenols in undegraded lignin will stain blue green, with clear zones around colonies indicating oxidation of phenolic compounds.

Tannic acid agar

LBM was supplemented with separately sterilized 1% w/v aqueous tannic acid solution. After inoculation, incubated at 25°C in darkness. Lignin degrading enzyme production is indicated by brown oxidation zone around colonies.

Guaicol agar

LBM with 1% guaicol was inoculated with the fungus. Development of pinkish red colour around the colonies indicate the release of laccase enzyme.

Identification of the selected fungi

Out of the 14 isolates, IS2 which exhibited good cellulase, xylanase and ligninase activity was selected for further studies. Its identification was done by Lactophenol cotton blue staining (James and Natalie, 2001). A drop of the stain was placed on a clean slide and with the aid of a needle a small portion of the mycelium from the culture plate was removed and placed in the drop of stain. With the help of the needle the mycelium was spread and a coverslip was placed over the stain. The slide was then observed under the microscope. It was identified further by 18S rRNA studies.

Results and Discussion

Isolation of fungi

A total of 14 fungi were isolated (IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS10, IS11, IS12, IS13, IS14). The isolated fungi were

maintained as pure cultures by sub culturing every four weeks.

Qualitative screening of isolated fungi for lignocellulolytic activities

Plate assays for all 14 isolates were carried out to know their potential cellulolytic, hemicellulolytic and ligninolytic activities. The isolates responded in different ways. Of all the isolates, IS2 had exhibited a promising cellulolytic, hemicellulolytic and ligninolytic activity. Qualitative screening for cellulose activity was observed as clear zones around the colonies, when the isolates were grown on CMC agar and stained with congo red. Out of the 14 isolates IS1, IS2, IS5, IS11 (Fig. 1) had shown clear zones indicating the cellulase activity. Xylanase activity was due to the degradation of xylan in the medium by the fungi. The activity was observed by clear zones around the colonies up on staining with grams iodine. Isolates IS1, IS2, IS5, IS6, IS7, IS8, IS9 (Fig. 2) showed clear zones which confirms the release of xylanase enzyme. Four different qualitative tests using Lignin, tannic acid, guaicol and azure B to the basal medium were conducted to detect the release of various ligninases. Basal medium with guaicol is test specifically for detecting the release of laccase enzyme. No isolate exhibited laccase activity. On the LBM plates the undegraded lignin remains blue green with clear zones around colonies indicating oxidation of phenolic compounds. IS1, IS2, IS7, IS8, IS9, IS10 (Fig. 4) were positive for lignin degradation. Browning of the medium around the colony in tannic acid containing medium indicates degradation of lignin (Fig. 3). IS5, IS7, IS9, IS10 exhibited browning of medium (Fig. 4). In Azure method, decolorization of the dye azure B indicates the production of LiP and MnP. Dye decolorization was shown only by IS2 (Fig. 5a). IS 11 could not completely decolorize Azure B (Table 1).

Table.1 Qualitative screening of the isolated fungi on CMC, lignin, Azure B, Guaicol, Tannic acid and xylan containing medium. (+) indicates release of the enzyme to degrade the respective substrate, (-) indicates no release of enzymes

	CMC	Lignin	Azure B	Guaicol	Tannic acid	Xylan
IS1	+	+	-	-	-	+
IS2	+	+	+	-	-	+
IS3	-	-	-	-	-	-
IS4	-	-	-	-	-	-
IS5	+	-	-	-	+	+
IS6	-	-	-	-	-	+
IS7	-	+	-	-	+	+
IS8	-	+	-	-	-	+
IS9	-	+	-	-	+	+
IS10	-	+	-	-	+	-
IS11	+	-	-	-	-	-
IS12	-	-	-	-	-	-
IS13	-	-	-	-	-	-
IS14	-	-	-	-	-	-

Fig.1 Endoglucanase activity of IS2, IS5, (a, b) showing zones of clearance and IS12 (c) showing no activity

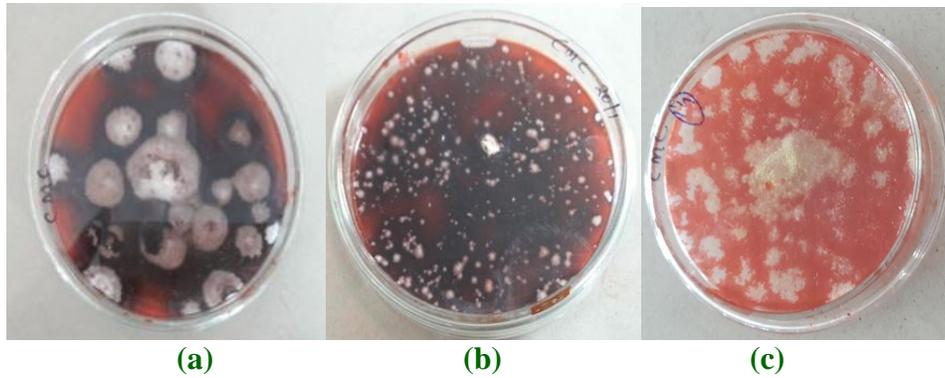


Fig.2 Ligninase activity of IS1, IS2 (a, b) showing zones of clearance a b c

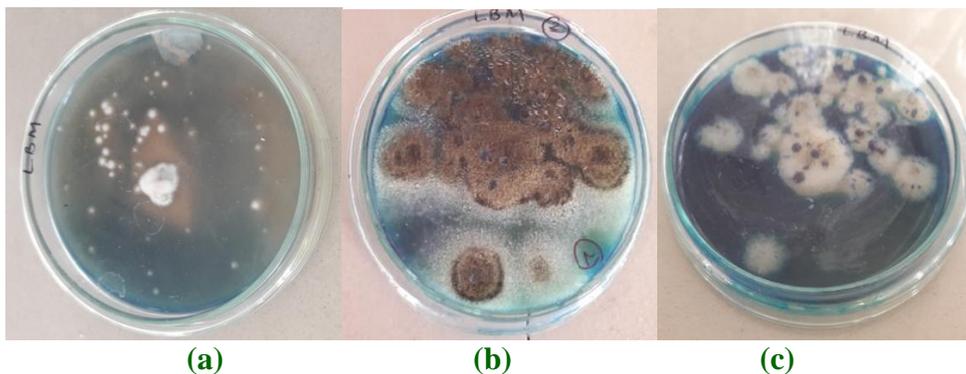


Fig.3 Ligninase activity of IS10, IS7 (a, b) on tannic acid medium showing browning of the medium, IS 11 (c) showing no browning

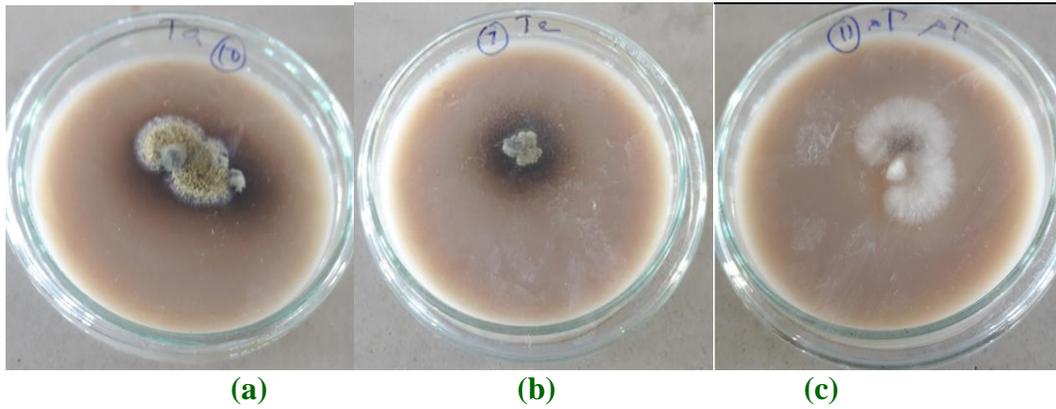


Fig.4 IS2(a) showing Azure B clearance, IS11 (b) showing partial clearance and IS5 showing no clearance of Azure B

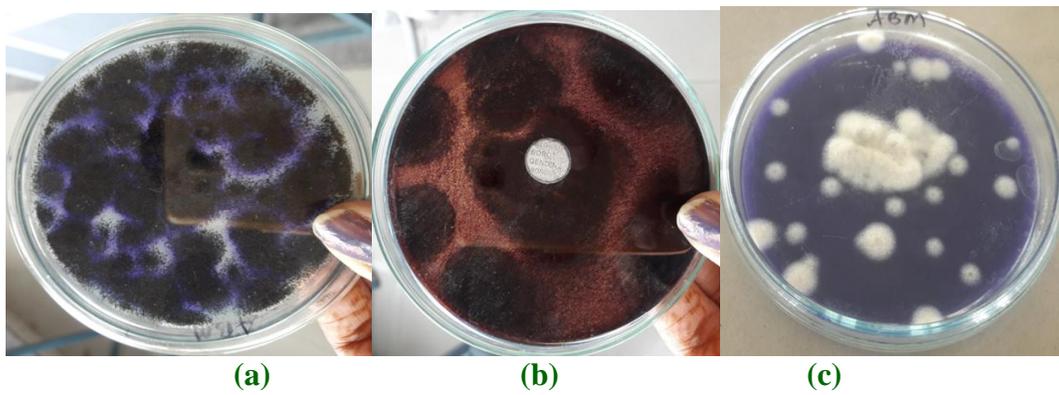


Fig.5 Xylanase activity of IS2 (a) showing zone of clearance and IS5(b) not exhibiting any clearance zone

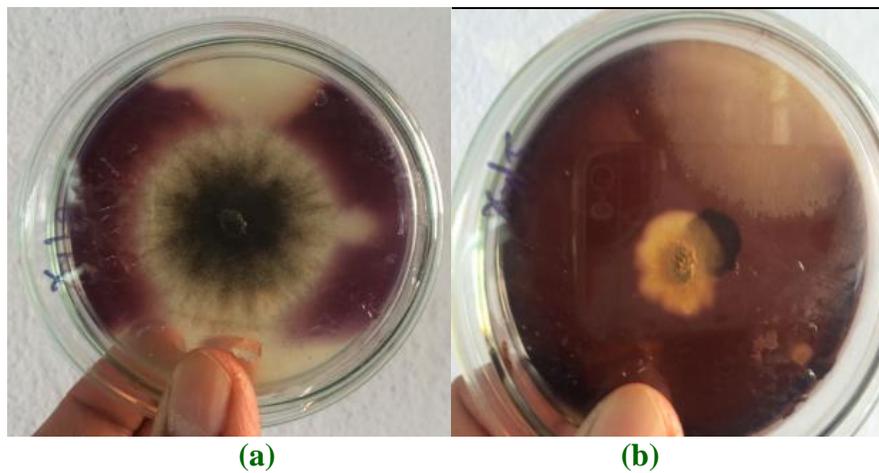
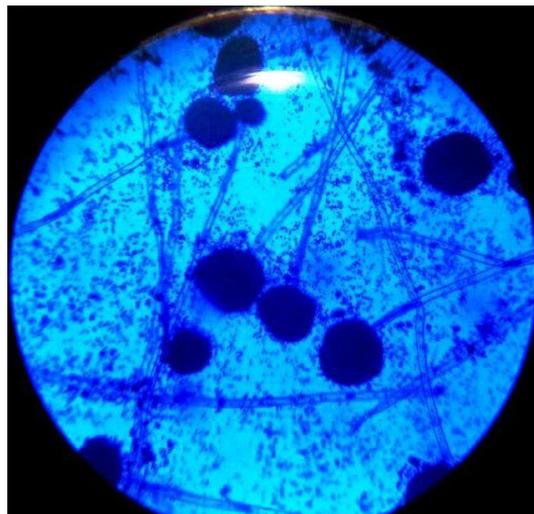


Fig.6 Microscopic view of IS2 up on staining with Lactophenol cotton blue stain



A partial degradation was indicated by development of pink colour of the plate (Fig. 5b) of the 14 isolates Isolate 2 (IS2) had shown a relatively good endoglucanase activity (Diameter of zone of clearance was more compared to other isolates positive for endoglucanase activity), Xylanase activity (Diameter of Zone of clearance is more) and peroxidase activity (Azure B clearance). Hence IS2 can be exploited for further studies.

Characterization and identification of Isolate 2

Up on staining with Lactophenol cotton blue stain, IS2 was identified as *Aspergillus niger*. Identification of IS2 was also done by 18s rRNA analysis. The screened IS2 was closely related to *Aspergillus niger* with 100% similarity.

There are several reports on the degradation of natural lignocellulosic substrates by *Aspergillus niger*. *Aspergillus niger* is already known to degrade sugarcane bagasse (Cunha *et al.*, 2012; Praveen Kumar Reddy *et al.*, 2015; Bhoosreddy, 2014), orange peels (Cunha *et al.*, 2012; Amaeze *et al.*, 2015),

pine apple peels (Amaeze *et al.*, 2015), coir waste (Soma Mrudula *et al.*, 2011), rice bran (Praveen Kumar Reddy *et al.*, 2015; Mahalakshmi *et al.*, 2016), saw dust (Praveen Kumar Reddy *et al.*, 2015; Bhoosreddy, 2014), ground nut shells (Praveen Kumar Reddy *et al.*, 2015), wheat bran (Praveen Kumar Reddy, *et al.*, 2015) and corn cobs (Praveen Kumar Reddy, *et al.*, 2015; Bhoosreddy, 2014).

No reports are available on the lignocellulosic degradation of fruit litter of *Albizia lebbek* Benth. Therefore *Aspergillus niger* can further be exploited for its capacity to degrade fruit litter of *Albizia lebbek*.

As this fungi exhibited a good cellulase, xylanase and ligninase activity, quantitative studies for the production of these enzymes can be carried out by both submerged and solid state fermentation.

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