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A Study on Isolation and characterization of Lignocellulosic Degrading Fungi from Cotton Crop Residues

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ABSTRACT: Lignocellulosic biomass is the most abundant biorenewable biomass on earth and is resistant to degradation. However, the presence of lignin in the biopolymeric structure makes it highly resistant to solubilization thereby hindering the hydrolysis of cellulose and hemicellulose. The utilization of biological methods to degrade lignocellulosic materials has been a very effective and eco-friendly method. The present study aimed to isolate and characterize lignocellulosic degrading fungi from different crop residues.

Totally 8 lignocellulosic degrading fungal colonies were isolated from 16 crop residue samples like cotton stalks from various locations. All 8 lignocellulosic degrading fungal colonies were confirmed as cellulose and lignin degraders through conformational test. The efficient lignocellulosic fungal cultures were identified based on morphological and cultural characterization and were tentatively identified as *Aspergillus* sp, Talaromyces and Fusarium sp. respectively, having significant potential for using them in the treatment of lignin and cellulose degradation.

Keywords: Lignocellulosic biomass, lignocellulosic degrading fungi, morphological and cultural characterization.

INTRODUCTION

Lignocellulosic biomass is an abundant and renewable resource from plants mainly composed of polysaccharides (cellulose and hemicelluloses) and an aromatic polymer (lignin) (Zoghlami and Paes 2019). The major components of these materials are cellulose 35%-50%, hemicellulose 20% - 35%, and lignin 10% - 25%. The remaining fraction of lignocellulosic biomass includes proteins, oils, and ash (Peng *et al*., 2010). These components are present in different quantities in different plant species. Cellulose is a linear polymer of glucose linked through α -1, 4 linkages and is usually arranged into microcrystalline structures, which are very difficult to dissolve or hydrolyze. Hemicellulose is a heteropolysaccharide composed of different hexoses, pentoses and glucoronic acid. Hemicellulose is more soluble than cellulose and is frequently branched. Lignin is a highly irregular and insoluble polymer made up of phenylpropanoid subunits, namely p-coumaroyl, coniferyl, and sinapyl alcohols thereby making the enzymatic hydrolysis of this polymer extremely difficult.

Annual production of biomass is estimated to be 1 × 1010 MT worldwide Saritha *et al*. (2012). In India, 5 × 108 t of agricultural residues are generated as waste Sarsaiya *et al*. (2019). Paddy straw is the most abundant lignocellulosic waste on earth. Rice plant approximately contains cellulose 30-40%, hemicelluloses 20-24%, lignin 10-13%, ash 5% and silica 13%. The bark of cotton stalks contains about 19.3% hemicellulose, 23.1 % lignin and 32.1% cellulose (Dong *et al*., 2014) whereas the chemical composition of pigeon pea wood is cellulose 34.26 ± 0.29%, hemicellulose 34.83 ± 0.68%, and lignin 17.99 ± 0.22% (Tanquilut *et al*., 2019). About 90 Mt of crop residues are burned on-farm. One tonne of straw on burning releases 3 kg particulate matter, 60 kg CO, 1460 kg CO_2, 199 kg ash and 2 kg SO_2 Disposal of crop residue by burning is often criticized for accelerating losses of soil organic matter and nutrients, increasing carbon emissions causing intense air pollution and reducing soil microbial activity (Beiderbeck *et al*., 1980). Lignocellulose decomposition is influenced by the structure and function of microbial communities. Studies have shown that lignocellulosic biomass can be broken down by contributions from several microorganisms (Kumar *et al*., 2008) with manifold fungal and bacterial genera giving rise to cellulolytic and hemicellulolytic enzymes (Chukwuma *et al*., 2020) under aerobic and anaerobic surroundings to achieve this. Although fungi are the main cellulase-producing microorganisms, most studies of biodegradation

processes have emphasized the role of fungi because of their capability of producing and secreting high amounts of enzymes (Maki *et al*., 2009).

MATERIAL AND METHODS

Collection of samples: Cotton stalk crop residue samples were selected to get maximum population of lignocellulolytic microorganisms from various locations. Crop residue samples were collected from different spots in sterilized polythene bags. The Polythene bags were properly tied, labeled and atmost care was taken to avoid contamination and were preserved in a deep freezer at 0ºC for further use.

Isolation of lignocellulosic degrading fungi from cotton crop residues : For isolation from crop residues ten grams of samples were weighed and serial dilution plate technique was carried out up to 10-6 dilution and appropriate dilutions (100µl) was spread plate using Potato Dextrose Agar (PDA) medium and Saubour and Dextrose Agar (SDA) medium supplemented with Carboxy Methyl Cellulose (1% CMC). Further plates were kept for incubation at 27±2ºC for 6 -7 days. Morphologically different colonies were selected for isolation and sub-culturing. Fungal colonies were picked carefully and maintained in plates.

Identification of fungal isolates by morphological and cultural characteristics: Potato dextrose agar (PDA) plates were inoculated with selected fungal isolates and incubated at 25 ± 2°C.

Morphological characterization : For morphological characterization, the cultures were incubated at 25 ± 2 °C. Microscopic preparations for morphological studies, slides were prepared by adding culture followed by lactophenol-cotton blue. After placing the coverslip, the slide was observed under the microscope for morphological characteristics like type of mycelium, phialides arrangement, conidial shape and colour. Species identification was based on the morphological and taxonomic keys provided by Bissett (1991).

Cultural characterization : For cultural studies, Petri plates were inoculated with pure cultures and incubated at 25 ± 2 °C. The colonies were examined at 24 h intervals for recording colony diameter, type of colony growth, and margin of colony.

RESULTS AND DISCUSSION

A total of sixteen cotton crop residue samples were gathered from various locations for the isolation of lignocellulosic degrading fungi.

Isolation of lignocellulosic degrading fungi from cotton crop residues : A total of sixteen crop residue samples were gathered from various locations for isolation of lignocellulosic degrading fungi. Among them, eight lignocellulosic degrading fungal isolates showed morphologically distinct growth on Potato Dextrose Agar (PDA) medium and Saubourand Dextrose Agar (SDA) medium supplemented with Carboxy methyl cellulose (1% CMC) as their sole carbon source. The fungal isolates showed positive for cellulase activity by the appearance of a clear zone around the colony indicating cellulase degrading capability. All the isolates were subsequently purified and preserved for further study (Fig. 1).

The results were found to be in confirmation with the findings of Lahiri *et al*. (2021) isolated 83 bacterial isolates and 18 fungal isolates from semi-rotten tissues of trees, organic matter including compost, vermicompost. BB12 and CCB9 isolates showed the highest cellulase activity with a ratio of halo diameter to colony diameter of 9.5 and 7.5 followed by fungal isolates WF2 and WF4 isolates 4.57 and 2.4.

Morphological and Cultural Characterization: Based on qualitative and quantitative screening, the efficient lignocellulosic fungal cultures were selected for identification. Morphological characterization includes the type of mycelium, phialides arrangement, conidial shape and colour. Septate and branched were observed. Phialides arrangement was flasked to bottle shaped. Further conidial shape varied from single-celled and oval, globose or elliptical and conidial colour varied from green, hyaline, white yellow and black, Based on morphological and cultural characterization fungal cultures were tentatively identified as *Aspergillus* sp, *Talaromyces*, and *Fusarium* sp. Cultural characterization usually includes colony diameter, colony color, margin of colony and type of colony growth. Colony diameter varied from 85-90 mm, different colony color was observed like green, greenish-yellow, white and black. Further margin of the colony was entire to irregular and the type of colony growth varied from cottony to fluffy.

Results were in tune with Nirmalasari *et al*. (2022) studied potential lignocellulolytic fungi isolated from different type cocoa cropping pattern. Among the 23 isolates, 4 isolates were identified as potential lignocellulolytic fungi by enzyme activities. Based on the morphology of isolates CSF#5 and CSF#15, it was known that the two isolates belonged to the *Ascomycetes* group strain due to the presence of insulated conidium and hyphae. A lignin and cellulose-degrading fungal strain Bio-1 was isolated by Jin *et al*. (2012) It was identified as a member of the genus *Cladosporium* by 18s rDNA, ITS sequences analysis and morphological characters such as the basic shape of the Bio-1 colony is circular with entire edge and wrinkled surface, the color of the fruit bodies is dark-green.

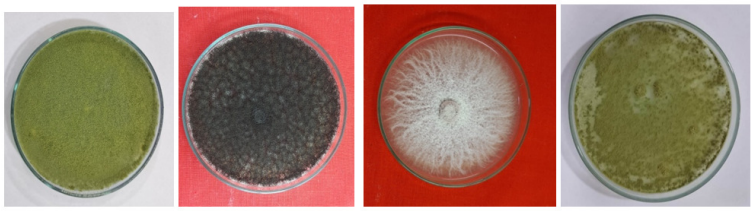

Fig. 1. Lignocellulosic fungal cultures isolated from cotton crop residues.

Fig. 2. Cellulase activity of lignocellulosic fungal isolates.

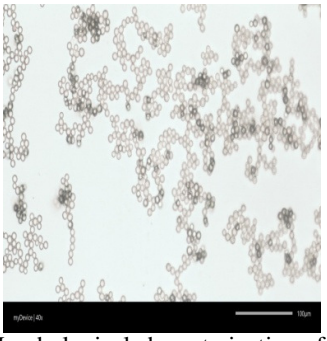

Fig. 3. Morphological characterization of efficient lignocellulosic degrading fungi.

CONCLUSIONS

In this present study, it can be concluded that cotton crop residues are a good source for the isolation of lignocellulosic degrading fungi and fungal isolates such as Aspergillus sp, Talaromyces, and Fusarium sp can be effectively used for the degradation of cellulose and lignin.

FUTURE SCOPE

In future, there is a scope of using lignocellulosic degrading fungi for many off-field options like composting and biogas production.

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Conflict of Interest. None.

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