

RESEARCH ARTICLE

Lignocellulolytic Potentials of *Aspergillus terreus* for Management of Wheat Crop Residues

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Abstract

The present study was carried out to assess the enzymatic potential of fungal species during decomposition of wastes. Study showed that *Aspergillus terreus*, was an efficient decomposer of wheat crop residues and it is also able to decompose the wastes *in vitro*. Also the ability of *Aspergillus terreus* to produce degradative enzymes viz. cellulase, hemicellulase, pectinases and ligninases *in vitro* was tested. The fungus showed good ability to decompose insoluble cellulose and lignin *in vitro* as assessed by its ability to grow on media containing insoluble cellulose and lignin. The fungus also possesses moderate activities to decompose carboxy-methyl-cellulose (CMC), xylan and pectin. These components are natural polymers found in all plant waste materials. The prerequisite of any strategy used for biotechnological management of plant wastes is to break down these polymers. The findings suggest the possibilities of utilizing *Aspergillus terreus* as an integral component of microbe-based strategies for management of organic wastes to clean and safe environment.

Keywords: *Aspergillus terreus*, lignocellulose, wheat, decomposer, degradative enzymes.

Introduction

Like many other agriculture wastes, wheat straw also largely consists of lignified cell wall material—cellulose, hemi-cellulose, lignin being the three major structural components. Out of these, cellulose is the predominant constituent followed by hemicelluloses and lignin (Charaya, 1985). Cellulose like starch is a polymer of D-glucose, but it differs from starch in having $\beta(1-4)$ linkages, while starch has $\alpha(1-4)$ linkages and this difference results in strong hydrogen bonding and gives cellulose its physical strength and rigidity (Anderson and Anderson, 1980). Lignin, together with hemi-cellulose, encrusts the cellulose chains forming a barrier which prevents wetting and action of cellulose degrading enzymes (Kirk and Haskin, 1973). To utilize the lingo-cellulosic components as a source of energy and other needs, this association has to be broken first. Any strategy aimed at efficient management of straw must involve following steps.

- Loosening of the association between lignin, hemi-cellulose and cellulose.
- Degradation of cellulose, hemi-cellulose and lignin.
- Further transformation for the utilization of various degradation products for human welfare.

Sanchez (2009) studied that the ability of fungi to degrade lignocellulosic materials is due to their highly efficient enzymatic system. Fungi have two types of extracellular enzymatic systems; the hydrolytic system, which produces hydrolases that are responsible for

polysaccharide degradation and a unique oxidative and extracellular ligninolytic system, which degrades lignin and opens phenyl rings. Feng *et al.* (2011) showed that presence of lignolytic enzymes could enhance the activity of microorganisms and it would be undoubtedly, worthwhile using selected microorganisms present in the straw naturally or those which possess suitable properties for loosening the lignocellulosic bond (Eggin and Seal, 1978). Potential benefits of using specific microbial strain in mineralization of straw have already been demonstrated by a number of workers including Koutev *et al.* (2001). It is in this context, that the study of lignocellulolytic potential of *Aspergillus terreus* decomposing wheat straw becomes important.

Materials and methods

Isolation and identification of test fungi: Isolation and identification of the test fungi was done in Lab of Microbiology, Dept. of Botany, CCS University, Meerut under the supervision of Dr. M.U. Charaya (Prof. & Head). One gram of the material (internode chaff/leaves/straw) was cut with flamed scissor into 5 mm pieces, placed in 100 mL of sterile water and stirred for 15 min to wash microbial propagules from the material. Ten mL of this suspension (1: 100 dilutions) were immediately transferred to a conical flask containing 90 mL of sterilized distilled water. This suspension was used for the preparation of further serial dilutions (1: 1000, 1: 10,000 and 1: 100,000) aseptically.

From suspensions of each of the last three dilutions, 1 mL aliquots were transferred to each of a set of three petri dishes, followed by the addition of 20 mL cooled and sterilized Czapek-Dox agar medium (Raper and Thom, 1949) with 30 ppm of Rose Bengal and Streptomycin. The petri dishes containing the medium and the inoculum were incubated at $25\pm 1^\circ\text{C}$ for 6-8 days. The incubated petri dishes were observed from the 3rd day itself when fast-growing forms of fungi made their appearance. The identification was done on the basis of their morphology and cultural characteristics following Subramanian (1971).

Ability to utilize insoluble cellulose: The method adopted by Fisher *et al.* (1977) was followed with slight modification to assess the ability of the test fungus to utilize insoluble cellulose. The test fungus was grown in Erlenmeyer flask (100 mL capacity), each flask containing 50 mL of the medium. A single, oven-dried (at 95°C), pre-weighed Whatman No. 1 paper disc was suspended in the medium with the help of a nylon thread. The flasks were sterilized by autoclaving at 121°C for 15 min. Agar-mycelial discs (7 mm) of the test fungus grown for 5 days at $25\pm 1^\circ\text{C}$ on Potato Dextrose agar medium were used as inocula and uninoculated flasks served as control. Flasks were incubated at $25\pm 1^\circ\text{C}$ for 3 weeks under stationary conditions and were harvested thereafter. At the time of harvest, the filter paper from each flask was removed and dried to constant weight at 80°C . A comparison of the dry weight of the filter paper at the end of the incubation period was made with its dry weight at the start. The mean percentage loss in the dry weight of filter paper was calculated with respect to test fungus and it was considered to reflect the cellulolytic portion of the respective fungal species; of course, these losses represent only the portion of cellulose actively respired by the fungus, but not the proportion actually converted into fungal substance.

Cx activity: The fungus was also screened for the production of Cx (CMCase) activity following the method proposed by Hankin and Ariagnostakis (1977). The pH of the medium was adjusted to 6.5. Fifteen mL of the medium were poured into petri dishes; these were, on solidification, centrally inoculated with the test fungi. The plates were incubated 5 days and then flooded with 1% aqueous solution of hexadecyl trimethyl ammonium bromide. The carboxy methyl cellulose in the medium was precipitated leaving a clear zone around the colony in otherwise opaque medium indicating the degradation of carboxy methyl cellulose.

Polygalacturonase (PG) and Pectin lyase (Pnl = PGTE) activity: Assessment of test fungi for PG and Pnl activity was done according to the procedure recommended by Hankins and Anagnostakis (1975), the method being similar to that for CMCase except that the medium contained pectin in place CMC.

The medium at pH 7.0 was used to detect Pnl activity while the same medium at pH 5.0 was used to detect PG activity.

Xylanase activity: Solution of xylan was prepared by dissolving it in hot water since it is practically insoluble in cold water. The solution was added to the Czapek's medium (without sucrose) so as to give a final concentration of 5% xylan in the medium. About 20 g of agar agar were also added to the medium, 15 mL aliquots of the medium were poured into sterile petri dishes which were inoculated with the test fungi as usual. After incubation at $25\pm 1^\circ\text{C}$ for 10 days, the fungal growth was recorded measuring the diameters of the fungal colonies. The experiments were carried in triplicates. The mean radial growth of a species on xylan-containing medium minus the growth on the medium without xylan was taken as an index of xylanase activity.

Lignolytic activity: A preliminary screening of the test fungi for lignolytic activity was done following Bavendam's (1928) test which is based upon the principle and assumption that lignolytic fungi can be detected by their reaction with tannic acid which they oxidise to a brown product. Modified Waksman's agar medium, as suggested by Agarwal (1969), was used for the purpose. The petri dishes containing above medium were inoculated with test fungus and incubated at $28\pm 1^\circ\text{C}$ for one week. At the end of the period, these were examined for brown coloration around their colony.

Results and discussion

About 50 fungi were isolated and identified from which *Aspergillus terreus* was evaluated for their enzymatic potential in this study. It was observed that *A. terreus* exhibited maximum ability to degrade insoluble cellulose and also found to possess very good lignolytic activity. Test fungi showed moderate activities of CMCase, Xylanase, PGTE and PG activities (Table 1). Emiazzi *et al.* (2001) have reported that *A. terreus* produced 250 CMCases. Charaya (1985) studied the potential of different fungal species to produce cellulolytic, pectolytic, hemi-cellulolytic and lignolytic enzymes including *A. terreus*. According to Macauley and Thrower (1966), the fungi capable of utilizing cellulose or pectin are important primary colonizers. Sharma and Khan (1978) have reported that some of the initial colonizers have short parasitic phase, which enables them to penetrate and establish in the fresh tissue before decomposition starts. Kendrick and Burgers (1962) found pectolytic activity to be responsible for initial colonization. Huang *et al.* (2010) reported highest lignolytic and good cellulolytic activities during lignocellulosic waste composting. The results suggest the possibility of utilizing *Aspergillus terreus* as an integral component of microbe based strategies for management of agricultural waste clean and safe environment.

Table 1. *In vitro* cellulolytic, hemicellulolytic, pectolytic and lignolytic capabilities of *Aspergillus terreus*.

Test fungi	Substrate/Activities					Lignin (Bavendam's reaction)*
	Insoluble Cellulose**	CMC*	Xylan	Pectin		
				PGTE*	PG*	
<i>Aspergillus terreus</i>	16.18±1.52	++	4.62	++	++	++++

*In terms of ranking as compared to control (qualitative test), ** In terms of mean percentage loss of substrate as compared to control.

Conclusion

The transformation of straw is required for composting, mushroom production, for the production of xylitol, methane, biogas and alcohol. This transformation of straw involves its degradation or decomposition, through the intervention of living organisms. A proper well planned strategy needs to be evolved for the effective management of highly useful resource. In the present study, *Aspergillus terreus* fungus was used to test lignocellulolytic potential to degrade the straw and produce desirable products.

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