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Fungi as Active Bio- decomposers for their Potential use in the Degradation of Agri-Waste

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Abstract: Fungi produce a wide variety of extracellular enzymes which helps them to break down a range of organic matter like crop residue and agri-waste, decomposing them, thereby regulating soil fertility and carbon-nutrient balance in a environmentally sustainable manner.

Present study aims at screening, isolating and identifying active fungal cellulose decomposers from natural substrates like wheat- straw and comparing their cellulase activity on liquid medium using cellulose powder.

Keywords: Fungi, Cellulose decomposition, crop residue, agri-waste, soil fertility

1. INTRODUCTION

Crop residue like cellulose rich wheat-straw, corn, rice straw, sugarcane bagasse and fruit peels etc., if properly managed can provide raw material for textile, paper, pulp and biofuel industry and prove to improve the commercial viability of agriculture. However, for want of alternate techniques available to the farmers crop residue management in an environmentally sustainable way continues to remain a challenge. Water availability restrictions in terms of timing of the year and commercial necessity of growing more than two crops in a year has made this problem all the more acute. The current practice of leaving agri-waste to decompose in yards or farmers burning it to clear their fields for sowing the next crop are both causing huge environmental damage and human health issues. One is extremely slow and limited in terms of commercial advantage and the other 'quick-fix' process of burning crop residue and agri waste destroys the litter layer, reducing the essential organic matter returned to the soil besides eliminating the organisms that inhabit the surface soil and litter layer.[1]

Using biological decomposition as means of efficient decomposition in which active fungi and bacteria enhance the decomposition of plant residue. Fungi play a major role as decomposers and recyclers releasing essential nutrients like nitrogen, phosphorus and trace elements from decaying matter, making them available for the next crop. Fungi present in the soil are key components of agri-waste decomposition process and add back macro and micro nutrients to soil fertility. This process of decomposition can be accelerated by selective addition of particular fungal and bacterial inoculants in the form of formulations. [6]

2. CELLULOSE DECOMPOSITION

Cellulose is most abundant natural bio-resource for the production of bio-based products and bio-energy. Cellulolytic micro-organisms play an important role by recycling cellulose.

Cellulose is a complex polymer but it occurs in the form of insoluble crystalline microfibrils, highly resistant to enzymatic hydrolysis. All microbes known to degrade cellulose produce many enzymes which act together either separately or in complex form. Bio- conversion of cellulose to soluble sugars and glucose is catalysed by a group of enzymes called cellulases. Main cellulase components are endo 1-4 beta-D-glucanase, exo-1-4 beta-D-glucanase and beta glucosidase. Cellulose induces extra-cellular cellulase activity in cellulolytic fungi. Two important substrates for measuring endo-beta 1-4 glucanase activity is a soluble cellulose derivative such as carboxy-methyl cellulose (Cx-cellulase activity) and endo-beta 1-4 glucanase activity (C-1 cellulase). [7]

One such effort has been made to identify and isolate active fungal bio-decomposers belonging to various groups of fungi and analyse their potential role in Agro-waste management .On the basis of their cellulase activity on wheat straw and cellulose powder, they have been categorized into various groups.[3]

3. METHODOLOGY

Present study aims at screening, isolating and identifying active fungal cellulose decomposers from natural substrates like wheat- straw and comparing their cellulase activity on liquid medium using cellulose powder, Whatman filter paper and in solid cultures on wheat straw (a natural ligno- cellulosic substrate) at weekly intervals for a period of 60 days.

Initial screening was based on the ability of fungus to grow on cellulose agar medium containing soluble cellulose such as carboxy- methyl cellulose (1 percent) as carbon source. Various fungi belonging to different groups were isolated in pure cultures at 25 degree Celsius. For the purpose of this study only twelve of these actively growing members were chosen (Table.1)

Cellulase activity of these forms were estimated following method of Mandel [4]. Various cultures were grown on 25 ml of cellulose broth kept in 100 ml flask. Flasks were removed at weekly intervals till 35th day to determine time for maximum cellulase activity and its pattern over a period in case of different fungi. At the end of incubation period, culture filtrates were obtained by passing the contents of the flasks through G-1 sintered glass filters. The filtrates were made to 50 ml with 0.05 M sodium citrate buffer (pH 5). Fungal culture filtrate was used as crude enzyme extract. Cellulase production in each case was assayed both for carboxymethyl cellulase activity (Cx) and filter paper activity (C-1).

Wheat straw of variety *Arjun vulgare* (4-10 mm size) were oven dried, crushed and taken as substrate after complete sterilization. In 100 ml flask, 0.5 g of substrate were taken and moistened with 10 ml distilled water and autoclaved for one hour. The flasks were inoculated with 10 mm diameter agar pieces cut from margins of seven day old cultures of various fungi on czapek's agar. These flasks were removed at weekly intervals till 35 th day for determining cellulase activity.

Culture filtrates from inoculated wheat straw were obtained as described. Twenty ml of 0.05M Sodium Citrate buffer was added to each flask and shaken for 30 mins. The contents were first passed through G-1 sintered glass filter and then centrifuged at 10, 000 x rpm for 5 minutes to obtain clear supernatant. The culture filtrate thus obtained was made to 50 ml in each case with buffer and kept at 4 degree Celsius till enzyme was assayed [5].

4. ENZYME ASSAY

Cellulase activity of each fungus was determined on two substrates viz carboxymethyl cellulose(CMC) and filter paper. For CMCase activity (Cx), 1ml of 1%CMC was inoculated with 1 ml of 1% of CMC was incubated with 1ml of culture filtrates for 30 mins. At 50 degree. For C-1 cellulase activity Whatman No1 filter paper (1X6cm) was used. To the filter paper 1 ml of each of culture filtrates and sodium citrate buffer was added and incubated at 50 degree for one hour. In both the cases reaction was stopped by cooling the tubes under tap water and assayed for reducing sugars with DNS reagent[2]. To each tube 3ml of DNS reagent was added. These were incubated at 100 degree for 15 min, and O.D was taken at 640 nm. Blank was represented by 1 ml of culture filtrate heated at 100 degree to denature the enzyme.

5. OBSERVATIONS

Table1: Cellulase assay (peak value-14th day) of some fungal isolates cultured on different substrates for growth (Cellulose powder-CMC, Wheat-Straw, Filter Paper-FP)
(Cellulase activity was expressed as mg sugar/ml culture filtrate/h which was converted to enzyme units. An enzyme unit is defined as the activity of enzyme in 1ml of the culture filtrate which will produce an amount of reducing sugar equivalent to 1mg of glucose when incubated for 30 min at 50 degree with 1 ml of CMC dissolved in 0.05 M citrate buffer (pH-5) or incubated or incubated for 60 min at 50 degree with Whatman No. filter paper)

Fungal Isolates	**Cellulose Powder**		**Wheat-Straw**	
	CMC	FP	CMC	FP
Chaetomium bostrychodes	0.63	0.23	0.86	0.72
C. globosum	0.59	0.22	0.63	0.21
C. succineum	0.54	0.17	0.62	0.18
Chrysosporium pannorum	0.53	0.15	0.58	0.18
Cunninghamella echinulata	0.02	NA	NA	NA
Fusarium oxysporium	0.52	0.14	0.60	0.18

Fungal Isolates	Cellulose Powder		Wheat-Straw	
Gymnascus reessi	0.14	0.06	0.15	0.06
Lophotrichus bifurchotrichus	0.15	0.05	0.14	0.06
Mucor hiemalis	0.12	0.02	0.12	0.02
Phoma hibernica	0.23	0.07	0.40	0.12
Rhizopus nigricans	0.10	0.01	0.15	0.02
Sordaria humana	0.44	0.12	0.50	0.1

Cellulase activity of the selected fungi was estimated in liquid culture using cellulose powder and on solid culture on wheat straw. All the fungi showed carboxymethyl cellulase (Cx) activity on both the substrates except *Cunninghamella echinulate* which showed very little activity on cellulose powder and no activity on wheat straw. However, cellulolytic activity of all fungi was highest on wheat straw.

Lophotrichus bifurcotrichus, and *Phoma hibernica* showed equally good activity on both the substrates. Gymnascus reessi, Lophotrichus bifurcotrichus, Mucor hiemalis and Rhizopus nigricans showed low activity on both the substrates

TABLE 2. Grouping of various fungi based on CMC activity(units/ml) on different substrates(cellulose powder and wheat straw)

Activity Range	Group A	Group B	Group C	Group D
Units/ml	(0.85-0.51)	(0.50-0.21)	(0.20- 0.50)	Nil
Cellulase Activity	High	Moderate	Low	None
Fungal Isolates	*Chaetomium bostrychodes*	*Sordaria humana*	*Gymnascus reessii*	*Cunninghamella echinulata*
	C.globosum	*Phoma hibernica*	*Lophotrichus bifurcotrichus*	
	C. succineum		*Mucor hiemalis*	
	Chrysosporium pannorum		*Rhizopus nigricans*	
	Fusarium oxysporum			

On the basis of maximum carboxymethyl cellulase activity test fungi were categorized under four groups viz. highly cellulolytic, moderately cellulolytic, low cellulolytic and non- cellulolytic (Table.2)

Chaetomium bostrychodes, *C.globosum*, *C.succinium*, *Chrysosporium pannorum* and *Fusarium oxysporum* belonged to highly cellulolytic group with cellulolytic activity ranging from 0.85 to 0.50 units/ml. *Phoma hibernica* and *Sordaria humana* were moderately cellulolytic (0.50-0.21 units /ml).

Gymnascus reessi, *Lophotrichus bifurcotrichus*, *Mucor hiemalis* and *Rhizopus nigricans* were low cellulolytic (0.20-0.05) whereas *Cunninghamella echinulata* showed no activity on wheat straw and a very little activity on cellulose powder hence put under non-cellulolytic (Table.2)

6. DISCUSSION AND CONCLUSION

Chaetomium bostrychodes and *C. globosum* showed high C1 cellulase activity whereas *Mucor hiemalis* and *Rhizopus nigricans* showed minimum activity. It has been observed that most of the cellulolytic microrganisms hydrolyse modified cellulose but only some of them are able to attack natural celluloses. These studies carried out in vitro can give some clue to the rate at which these fungi will decompose other substrates of similar nature.

All the tested fungi showed peak value on the 14th day and became constant after 28 days of incubation. Such decline in cellulase activity has been suggested to be due to accumulation of sugars. The catabolic repression of cellulases whether inducible or constructive in nature is well established has also explained increased fungal cellulolytic rate based on catabolic repression. Catabolic repression of tends to keep the rate of consumption of insoluble carbon reserves of a cellulosic substrates at an economic level for the fungal colonizers.

For analysing the rate of decomposition of a substrate there are various other parameters should be taken into consideration. It was observed that Mucorales in general had high rate of growth but showed low cellulolytic activity as compared to the selected members from other groups. Mycelial growth was not related to cellulose degradation and varied among the different fungal groups.

Cellulase activity of various fungi estimated in liquid

(cellulose powder) and solid cultures (wheat straw) revealed that fungal response varied with the substrate-the source of cellulose. Among the members of Mucorales viz *Mucor hiemalis*, *Rhizopus nigricans* were observed to be cellulolytic except *Cunninghamella echinulata.* Their activity was low as compared to other members of the various groups.

There are a variety of bio-decomposers which are capable of depolymerising cellulases which hydrolyze ligno-celluloses of plant residues. These enzymes find use in Food, textile, pulp and paper industry. Market value of these microbe based decomposers will be increasing in the years to come [6].

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