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Isolation and Screening of Cellulolytic Fungi

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Abstract: *A large number of microorganisms are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of enzymes capable of completely hydrolyzing cellulose. Fungi are the main cellulase producing microorganisms. In this purposed study, four fungal species belonging to two genera i.e. Trichoderma and Aspergillus were isolated from different sources, screened and compared for their ability to degrade cellulose. The plate screening assay recommended by International Union of Pure and Applied Chemistry (IUPAC) were used in the investigation. Cellulolytic fungi were evaluated after 7 days for the production of cellulolytic enzymes by staining with 1% congo red. The diameter of clear zone on fungal plates, gave an approximate indication of cellulase activities.*

Keywords: *Cellulase enzyme, Cellulolytic fungi, Trichoderma reesei, Aspergillus niger, Aspergillus fumigates, Aspergillus flavus, etc.*

I. Introduction

Cellulose the primary structural component of plant cell wall. Cellulose is a linear polymer of D-glucose residue linked by β (1-4) glycosidic bonds. In plant cell wall, the cellulose fibers are embedded in and cross linked by a matrix of several polysaccharide that are composed of glucose as well as other monosaccharides. The microorganisms that secrete a series of enzymes collectively known as cellulase enzyme, capable of hydrolyzing the β (1-4) linkages of cellulose [1]. Cellulase is a multienzyme system composed of several enzymes with numerous isoenzymes, which act in synergy. The basic enzyme for the process of depolymerization of cellulose requires three types of enzymes: Endoglucanase, hydrolyses internal β (1-4) glucan chain of cellulose at random, Exoglucanase, removes cellobiose from the non- reducing end of cello-oligosaccharide and beta glucosidase, hydrolyses cellobiose to yield two molecules of glucose which completes the depolymerization of cellulose. Cellulases have enormous potential in industrial application [2]. Cellulase have been used from several years in food processing, feed preparation, waste water treatment, detergent formulation, textile production and in other areas. Additional applications include the production of wine, beer and fruit juice.

II. Materials And Methods

2.1 Isolation of fungi from soil:

In this experimental study, fungi were isolated from soil collected from different places (i.e. from saw mill, Ballarsha paper mill, seminary hills and Bazar gaon) and from termite. Serial dilution method was carried out for isolation of pure culture. The isolates were further inoculated on sterile PDA (potato dextrose agar) plates by point inoculation and incubated at 28^0 C for 48 hours in order to obtain pure fungal plates. These fungi were then subcultured and preserved in pure form. Colonial morphology and microscopic examinations of the various isolates of pure cultures were used to determine the reproductive and vegetative structures [3].

2.2 Characterization of isolated pure culture:

Place the drop of lactophenol cotton blue stain on a clean microscopic slide. Add a drop of lactophenol cotton blue on the slide. In an aseptic condition, take the hyphae using inoculating loop from the slant and streak on the slide. Cover the entire preparation with a clean cover slip. The slide is ready for the examination under the microscope.

2.3 Clear zone test for the identification of cellulolytic fungi:

Czapek medium (minimal medium) is used for this test. 1g of cellulose powder and 2g of agar powder were added in 100ml minimal medium (except glucose) in 250ml conical flask. After autoclaving and cooling up to 45^0C the medium was poured onto the autoclaved petriplate. After solidification, wells were made on agar plate by using carborer. 20 µl of spore suspension were loaded into the wells, and then incubated at 30^0 C for 4 to 5 days. The plates than flooded with 1% congo red dye (30min), followed by destaining with 1MNaCl solution for 20min. Diameter of clear zones were than measured[4].

2.4 Production of cellulase enzyme:

The minimal medium containing 2g of substrate (wheat bran, wheat husk, gram husk and carboxy methyl cellulose) as a sole source of carbon was used for enzymes production. One ml of fungal culture was inoculated into 100 ml of sterile medium the culture were incubated in shaking incubator for 4 days at room temperature [250C – 30 0 C] and from the 4th day cellulase activity was measured. Cultures were harvested by centrifugation at 6000 x g for 15 min and the cell free culture supernatant used as crude enzyme source [3].

2.5 Cellulase activity assay:

The cellulase activity of each culture was measured by determining the amount of reducing sugars liberated by using a dinitrosalicylic acid (DNSA) method . One unit of FP – ase , CMC – ase was defined as the amount of enzyme , which released µmole of reducing sugar measured as glucose per min under the assay condition [1].

2.6 Filter Paper Assay:

Filter paper assay (FPase) for total cellulase activity in the culture filtrate was determined according to the standard method. An Aliquot of 0 .5 ml of cell – free culture supernatant was transferred to a clean test tube. Add 1 ml of sodium citrate buffer (pH 4.8) and Whatmann no # 1 filter paper strip (6 x 1 cm) was immersed in 1 ml of 0.5 M Sodium citrate buffer of pH 5.0 . After incubation at 50 C for 1 hr, the reducing sugar released was estimated by dinitrosalicylic and (DNSA) method. Add 10 ml of distilled water to each tube, tubes were mixed and absorbance were noted at 550 nm in colorimeter .One unit of filter paper (FPU) activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar from filter paper per ml per minute[5,6].

2.7 Screening of fungal isolates:

Screening of fungi isolated on CMC agar after treatment with congo red showing clear zones around the colonies. Screening of cellulase producing fungi was performed on CMC agar plates flooded with congo red and washed with NaCl. Depending on the diameter of clear zone around the colony, three fungal isolates were identified as cellulase producing fungi and its initial identification was done by fungal staining and colony morphology[7,8]. The *Trichoderma* show maximum clearance around the colony (Table 1)[3,9]

III. Results And Discussion

Although ten to twelve fungal organisms were isolated from the soil and two from termite extract, only three from soil and one from termite were found to produce cellulase enzyme as was evident from clear zone test. They were identified in the University Department ofBotony, RTM Nagpur University, Nagpur. In this experimental work, different carbon sources were used such as Sorghum straw, Carboxymethyl cellulose and Cellulose powder. Data obtained after analysis in this experimental work have given in table 1, 2 &3. In the Solid state fermentation where Sorghum straw was used as a substrate or carbon source, different isolated fungi had shown their celluloytic activity. In the comparative study of their celluloytic activity, it was found that the *Trichoderma* gave higher cellulolytic activity [31.3 EU/mI /min] than the other three fungi .While the *Aspergillus niger* [26 .6 EU /mI /min] and *Aspergillus fumigatus* [25 EU /ml / min]gaves little bit similar activity .*Aspergillus flavus* gave least activity as compared to other three [13 .3EU / mI /min]. The high peak value of enzyme activity was found on 4^{th} day while it started declining from 5^{th}day onwards. In the Minimal medium, carbon sources such as Carboxymethyl cellulose and cellulose powder were used except glucose .The enzyme activity in case of CMC was found to be higher than in case of cellulose. It has been found that the high peak value of enzyme activity is shown on 8^{th}day while the activity goes on declining from the 9^{th}day onwards on both the cases. *Trichoderma* gave higher activity than the other three fungi. Screening test in which a clear zone test were performed, the data is given in table 1. From this study, it is concluded that the Sorghum straw is the best substrate or source for cellulase production.

Table: 1 Results of clear zone test

Sr. no.	Cultures	Diameter of zone in cm
1	*Trichoderma*	2.0
2	*Aspergillusniger*	1.5
3	*Aspergillusfumigatus*	1.2
4	*Aspergillusflavus*	0.5

Table: 2. Readings of fig: 1

Day	***Trichoderma sp.***	***Aspergillus niger***	***Aspergillus fumigates***	***Aspergillus flavus***
	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)
3rd	28.3	26.6	25	13.3
4th	31.3	29	28.3	8.3
5th	25	23	21.6	5.0
6th	18	16	10.8	3.3
7th	10	7.5	5.8	1.6
8th	3.3	1.6	1.6	0.0

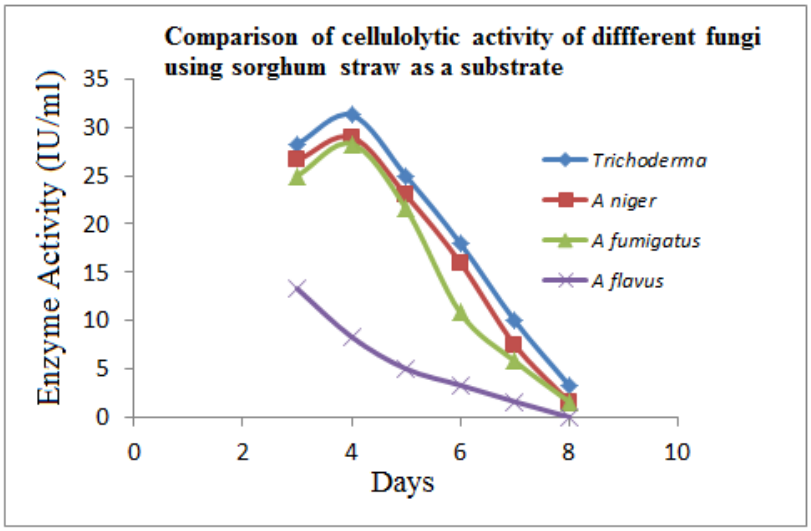

Fig: 1. Graph of enzyme activity of different fungi

Table: 3. Readings of Fig: 2

Day	***Trichoderma sp.***	***Aspergillus niger***	***Aspergillus fumigatus***	***Aspergillus flavus***
	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)
6th	11.6	10	10	3.3
7th	13.3	11.6	11.6	6.6
8th	15.0	15.0	13.3	10.0
9th	13.3	11.6	11.6	8.3
10th	10.0	10.0	8.3	6.6
11th	6.6	6.6	5.0	3.3
12th	3.3	3.3	3.3	1.6
13th	1.6	1.6	1.6	0.00

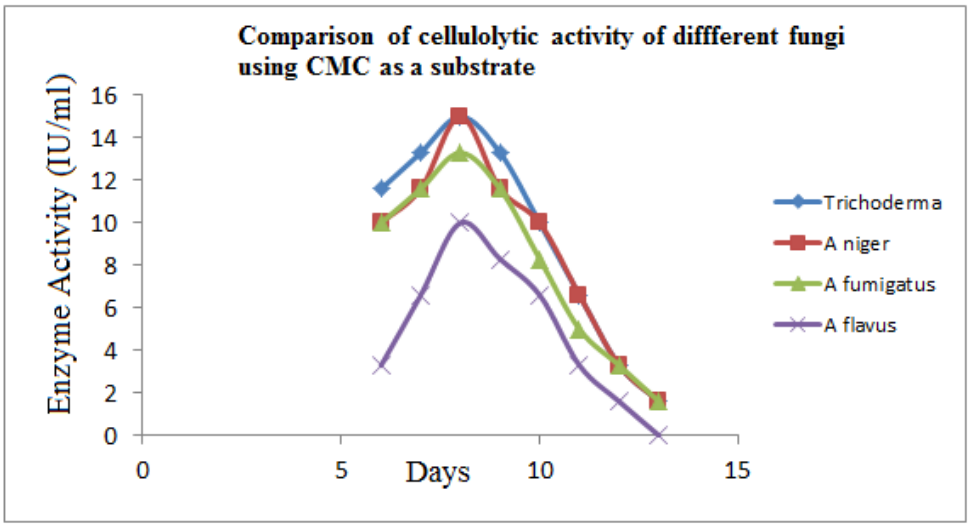

Fig: 2. Graph of enzyme activity of different fungi

Table: 4. Readings of fig: 3.

Day	***Trichoderma sp.***	***Aspergillus niger***	***Aspergillus fumigatus***	***Aspergillus flavus***
	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)	Enzyme activity in (U/ml/min)
6th	5.0	3.3	3.3	1.6
7th	8.3	5.0	5.0	3.3
8th	13.3	10.0	8.3	5.0
9th	15.0	11.6	8.3	6.6
10th	11.6	8.3	6.6	3.3
11th	10.0	6.6	3.3	3.3
12th	6.6	5.0	1.6	0.00
13th	3.3	1.6	0.00	0.00

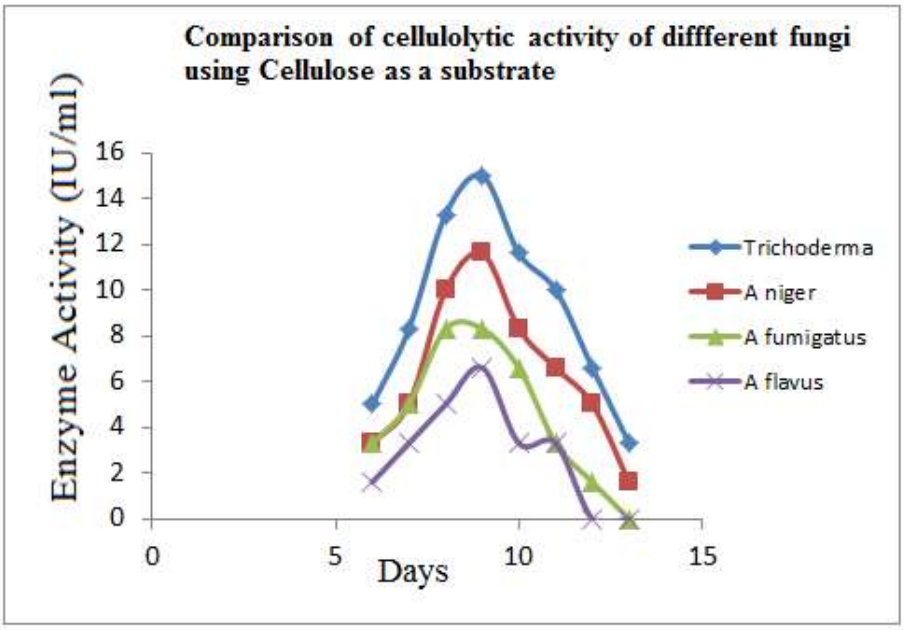

Fig: 3. Graph of enzyme activity of different fungi

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