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ORIGINAL RESEARCH ARTICLE

Isolation and Characterization of Rapid Cellulose Degrading Fungal Pathogens from Compost of Agro Wastes

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ABSTRACT

Cellulose is the most abundant biopolymer renewable natural product in the biosphere. Cellulose degrading fungal pathogens play an important role in the biosphere by recycling cellulose mediated by cellulase enzyme and common in field such as forest soils, in manure and on decaying plant tissues. This present study was focused on the isolation, identification and strain improvement of cellulose degrading fungus from compost of agro wastes. 0.1 g of compost samples from 10^{-3} and 10^{-4} serially diluted and cultured on Rose Bengal Agar medium (RBA) enriched with 1% Carboxy Methyl Cellulose(CMC) as sole source of carbon. Cellulose degrading fungal pathogens were identified by standard phenotypic method. Isolated cellulose degrading fungal pathogens were subjected with UV-irradiation method at different times of intervals. All exposed RBA with 1% CMC plates were incubated at maximum period and observed fungal growth pattern. Strongly UV resistant cellulose degrading fungal mutant strain and wild strain was evaluated and confirmed by reducing sugar property method. From this study, six pure potential cellulose degrading fungal pathogens (i.e., *Fusarium* sp., *Aspergillus fumigatus*, *Cladosporium* sp., *Aspergillus flavus*, *Pyricularia* sp. and *Nigrospora* sp.) were isolated. Out of six UV exposed strains of cellulose degrading fungus, Fusarium genus only showed excellent growth at 5th day of incubation at different time of intervals. While other five strains of cellulose degrading fungus were showed no growth at maximum period of incubation. This study was concluded that the mutant strain of *Fusarium* genus was considered as an effective strain for cellulose degradation. Hence it was suggested that, this mutant strain could be exploited for composting of various organic wastes.

Key words: Cellulose, Hemicellulose, Lignin, Compost, Rose Bengal Agar, Carboxy Methyl Cellulose and *Fusarium* sp.

1. INTRODUCTION

Compost is a fertilizing mixture of partially decomposed organic matter from plant and animal origin. Various biological studies have been carried out to identify the microbiological agents responsible for biodegradation [1,2]. Cellulose degrading microorganisms play an important role in the biosphere by recycling cellulose and common in field such as forest soils, in manure, and on decaying plant tissues. Among the cellulose utilizing species are aerobic, anaerobic, mesophilic and thermophilic bacteria, filamentous fungi, basidiomycetes and actinomycetes [3,4]. A diverse group of fungi utilizes cellulose for its carbon and energy sources. Following treatment of soil with cellulose, there is a significant increase in the number of fungi, particularly if the nitrogen supply is adequate.

Plate count of filamentous fungi in excess of 10^6 per gram of soil during the decomposition of straw plus $NaNO_3$ are not common [5]. Strongly cellulose degrading fungi are represented by species of the genera *Aspergillus chaetomium*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Curvularia*, *Fusarium*, *Memoniella*, *Phomo*, *Thielavia* and *Trichoderma*. These strains have been extensively studied in their ability to produce extracellular cellulose degrading enzymes namely endoglucanases, exoglucanases and cellobiase which act synergistically the conversion of cellulose to glucose [6]. In general the wild strains of cellulolytic fungi produce low quantities of

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commercially important metabolites, although the yield can be increased by optimizing the fermentation conditions. The potentially of the metabolite formation is genetically determined. Therefore, genetically improvements have to be made and new cellulolytic fungal strains developed for any substantial increase in product formation in a cost effective manner [7,8]. The present study was aimed to isolate, identify and strain improvement of cellulose degrading fungus from compost of agro wastes.

2. MATERIALS AND METHODS:

The compost sample prepared from agro wastes was collected from the Department of Biology, Gandhigram Rural University, Gandhigram, Dindugal District, Tamilnadu. Pure compost samples were taken and serially diluted by standard method. 0.1 g of compost samples from 10^{-3} and 10^{-4} dilutions were taken and spreaded on Rose Bengal Agar medium (RBA) enriched with 1% Carboxy Methyl Cellulose (CMC) as a sole source of carbon for isolation of cellulose degrading fungus. Additionally 30 mg of streptomycin was incorporated in 1000ml of this medium for inhibiting the growth of other bacteria. All the plates were incubated at room temperature for 2-5 days. After incubation, according to their colony characteristics and zone of clearance indicated cellulose degrading fungus from mixed fungal population on primary culture plate of RBA with 1% CMC plate. The cellulose degrading fungus was picked out from primary culture plate and again sub cultured on RBA with 1% CMC plate incubated at room temperature for 2-5days. After incubation, the pure cellulose degrading fungal strains were spotted in the Rose Bengal Agar slant for genus confirmation. All the cellulose degrading fungus was identified by Lacto Phenol Cotton Blue (LPC) staining method and the results were recorded.

The pure cellulose degrading various genus of fungal pathogens were inoculated in to RBA with 1% CMC plate exposed to UV-irradiation method at different time intervals (5 min, 10min, 15min, and 20min) in Biological Safety Cabinet II (BSC-II). Before this work the Biological Safety Cabinet was cleaned by disinfectant and also put one control plate for checking the contamination for every time. Quality control was satisfied and work has been carried out in the BSC-II. All UV exposed fungal plates were incubated at room temperature and observed the range of growth parameters on every day for upto 5 days.

Estimation of reducing sugars released by wild and mutant strain of cellulose degrading fungus of *Fusarium* genus.

The Czapeck`s mineral salt broth was prepared at different concentrations with CMC (0.25%, 0.50%, 0.75%, 1.0%). The wild and mutant cellulose degrading fungal culture was inoculated into the conical flask. Every 24 hours, samples of wild and mutant cellulose degrading fungal cultures were collected from the culture flask and analyzed cellulose degradation by estimating the reducing sugar property by Somagyi-Nelson method [9].

3. RESULTS AND DISCUSSION

From this study, six pure potential cellulose degrading fungal pathogens (i.e., *Fusarium* sp., *Aspergillus fumigatus*, *Cladosporium* sp., *Aspergillus flavus*, *Pyricularia* sp. and *Nigrospora* sp.) were isolated on RBA plate enriched with 1% CMC showed zone of clearance around their colony (**Fig 1**). According to their zone of clearance, which has been sub cultured on RBA plate with 1%CMC and again confirmed by cultural characteristics and microscopic identification by Lactophenol cotton blue staining method (**Fig 2 & Table 1**)

The above six cellulose degrading fungal isolates were subjected with UV irradiation at different time intervals (i.e., 5, 10, 15 and 20 minutes) and incubated the RBA with 1% CMC plate at room temperature. After incubation, out of six UV exposed strains of cellulose degrading fungus, *Fusarium* genus only showed excellent growth at 5^{th} day of incubation at different time of intervals. While other five strains of cellulose degrading fungus were showed no growth at maximum period of incubation (**Fig 3**). Based on growth performance, the mutant *Fusarium* was selected for evaluating its efficacy on cellulose degrading activity along with its wild strain. Both mutant and wild strain of *Fusarium* genus were separately inoculated into the Czapeck`s mineral salt broth supplemented with different concentration of CMC (i.e., 0.25%, 0.50%, 0.75%, 1.0%). After incubation the reducing sugar released from each cultured flask of different concentration were estimated at every 24 hours. Both mutant and wild strain of *Fusarium* genus showed better activity in 0.25% CMC but as the concentration of CMC increased from 0.5 to 1.0% which has been increased release of sugar in the mutant inoculated culture flask. *Fusarium* mutant starts degrading Carboxy methylcellulose from day of incubation and showed the maximum cellulose degrading

activity on second day in concentration 0.75% with a released of 0.09mg/ml of reducing sugar when compared with its wild strain.(**Table 2 & 3**). Similar kinds of results were already reported by several review of literature. A number of other groups of fungi *Trichoderma koningii, Sporotrichum thermophila, Myceliophthora thermophila* have reported to possess cellulose degrading activity [10].

Cellulose degrading bacteria is another corner stone and great significant in manure, agro residues and semiarid localities. The numbers of aerobic, mesophilic bacteria metabolizing cellulose vary enormously from location to location, sometimes being less than 100 and sometimes more than 10 million per gram. The abundance is for greater in manured fields and sometimes in proximity to plant roots. *Cellulomonas*, *Cytophaga*, *Sporocytophaga* and other *Myxobacteria* classified as species of *Angiococcus* and *Polyangium* have showed significant role in cellulose degradation at various environmental origin [11, 12]. Other bacterial pathogens such as *Pseudomonas, Vibrio* and *Bacillus* are utilized little amount of cellulose source in the culture medium than the fungal pathogens. Actinomycetes is an another important group of the poor microbial community responsible for nutrient recycling in natural substances. Actinomycetes that gown on cellulose have received little attention despite their presence during the decay of cellulosic materials [13].

Table 1 Colony characteristics and Microscopic identification of cellulose degrading fungal isolates from compost of agro wastes.

Fungal isolate number	Colony appearance on RBA with 1%CMC plate	Microscopic appearance of fungal elements by LPC staining method	Final results
1	White to pink; woolly cottony appearance	Sickled shaped transversely septate macro conidia produced in sporodochia	*Fusarium* sp.
2	Greenish –blue black or green colonies	Conidiophores arising from a foot cell catenate (Basipetal) conidia on phialides (1or 2 series) on vesicle.	*Aspergillus fumigatus*
3	Greenish black and powdery appearance	Branched conidiophores; conidia variable	*Cladosporium* sp.
4	White powdery; yellow colour spores.	Septate; hyaline; conidial heads were radiated.	*Aspergillus flavus*
5	Black to grey; cottony appearance	Septate hyphae, oval shaped and septate conidia	*Pyricularia* sp.
6	White to grey color appearance	Unicellular dark colored spherical conidia	*Nigrospora* sp.

Table 2: Evaluation of cellulose degrading activity against wild strain of Fusarium genus by Somagyi method.

Type of fungal strain	Different concentration of CMC (%)	Concentration (mg/ml) Number of days					
		0th	1st	2nd	3rd	4th	5th
Wild strain of *Fusarium* genus	0.25	0.01	0.026	0.010	0.095	0.04	0.05
	0.50	0.04	0.014	0.021	0.021	0.015	0.014
	0.75	0.03	0.014	0.011	0.03	0.010	0.011
	1.0	0.033	0.020	0.013	0.034	0.044	0.069

Table 3: Evaluation of cellulose degrading activity against mutant strain of Fusarium genus by Somagyi method

Type of fungal strain	Different concentration of CMC (%)	Concentration(mg/ml) Number of days					
		0th	1st	2nd	3rd	4th	5th
UV-Resistant strain of *Fusarium* genus	0.25	0.017	0.016	0.014	0.010	0.09	0.017
	0.50	0.05	0.07	0.011	0.09	0.011	0.010
	0.75	0.045	0.010	0.09	0.01	0.011	0.0145
	1.0	0.025	0.012	0.033	0.056	0.079	0.080

Fig 1: Mixed fungal population along with cellulolytic fungus also isolated on Rose Bengal Agar enriched 1%Carboxy Methyl Cellulose from compost of agro wastes.

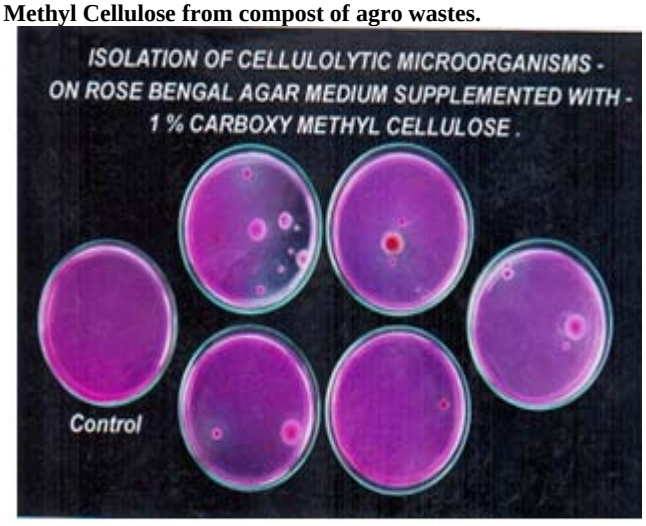


Fig: 2 Colony morphology and zone of clearance indicated (six fungal pathogens) cellulose degradation on RBA with 1% CMC plate.

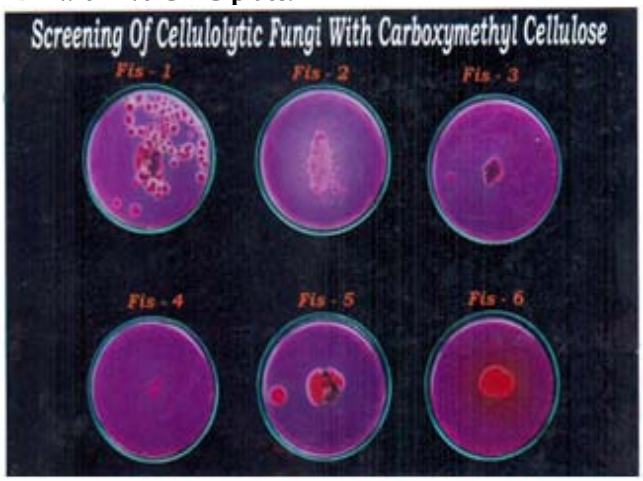
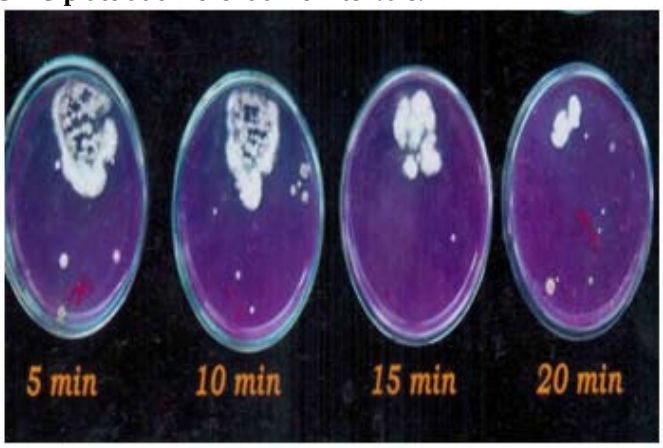


Fig:3 UV resistant Fusarium genus on RBA with 1% CMC plate at different time intervals.



4. CONCLUSION

Cellulose rich plant biomass is one of the foreseeable and sustainable source of fuel, animal feed and feed stock for chemical synthesis. The utilization of cellulosic biomass continues to be a subject of worldwide interest in view of fast depletion of our oil reserves and food shortage. Cellulose degrading fungus produced enzymes, which are similarly related to xylanases. Both enzymes are synergistic over substrate, especially for microorganisms isolated from environments where wood and agro residues are biodegraded. Based on the above discussion the mutant strain of *Fusarium* genus was released higher quantity of reducing sugar in the culture medium. So, improved fungal strain is to be needed for fast composting process.

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