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RESEARCH ARTICLE

ISOLATION, IDENTIFICATION AND SCREENING OF CELLULOSE DEGRADING MICROFUNGI FROM DECOMPOSED *E.CRASSIPES*

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ABSTRACT

Water Hyacinth (*Echhornia crassipes*) is the most important aquatic weeds. This study aimed to identify the species of microfungi which were grown in soil and degraded cellulose present in *E. crassipes*. Different types of fungi were isolated from soil samples containing decomposed *E. crassipes*. Soil containing decomposed *E. crassipes* from Laxmi Taal, Jhansi was investigated. Isolation of microfungi from soil was carried out on Potato Dextrose Agar (PDA) and microscopic method. A total of fourteen (14) microfungi was isolated namely *Alternaria, Aspergillus niger, Cladosporium, Curvularia, Fusarium, Mucor, Nigrospora, Phoma, Pythium, Rizhopus, Gliocladium, Penicillium, Trichoderma, Helminthosporium*. Of these microfungi *Trichoderma, Mucor, Fusarium* and *Aspergillus* had great potential for growth on PDA. Cellulose is the most abundant biopolymer renewable natural product in the biosphere. The present study was also focused on identification and screening of cellulose degrading fungi from samples of Water Hyacinth wastes. Dominant isolates of cellulase producing fungi were isolated from growth culture. The samples were grown in Potato Dextrose Agar medium containig 1% carboxymethylcellulose (CMC) sodium salt. Clear zones surrounded the colonies with zone diameter measuring 1.2 to 4.5 cm. On the basis of morphological characteristics the isolates were identified as *A. niger . Penicillium spp*, *Trichoderma spp and Fusarium spp., Chaetomium, Rhizopus* but *Aspergillus* and *Trichoderma* were more potentially cellulose degrading microfungi deu to more clear zone diameter.

Key Words: Cellulose, Carboxy Methyl Cellulose, Microfungi , Potato Dextrose Agar, Water Hyacinth, *Trichoderma.*

INTRODUCTION

Traditional biologists have defined fungi as eukaryotic, spore pruducing , achlorophyllous organism with absorptive nutrition that generally reproduce both sexually and asexually and usually whose filamentous, branched, somatic structure known as hyphae typically surrounede by cell wall. Fungi play an important role in our ecosphere as agent of decay and as a principal agent that decay cellulose and lignin. Microfungi as a decomposer play an important role in the decomposition of biological organic materials. Many along with pathogenic organisms are present in litter even at very early stage (Hudson 1962, Lindsey and Pugh 1976).

At present there is a worldwide search for suitable microfungi which are capable of degrading biomass. In addition, some research is known to have been carried out to investigate the possibilities for large-scale production of these microfungi (Mahro *et al.,* 1994; Field *et al.,* 1996; Anon, 1998). Various biological studies have been carried out to identify the microbiological agents responsible for biodegradation (Singh, 1987: Lyand *et al.,* 2002). Strongly cellulose degrading fungi are represented by species of the genera *Aspergillus Chaetomium sp.*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Curvularia*, *Fusarium oxysporum*, *Memoniella*, *Phoma*, *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 (Maheshwari *et al*., 1990).

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Cellulolytic enzymes are synthesized by a number of microorganisms. Fungi and bacteria are the main natural agents of cellulose degradation (Lederberg, 1992). The cellulose utilizing population includes aerobic and anaerobic mesophilic bacteria, filamentous fungi, thermophilic and alkaliphilic bacteria, actinomycetes and certain protozoa (Alexander, 1961). However, fungi are well known agents of decomposition of organic matter, in general, and of cellulosic substrate in particular (Lynd *et al*., 2002). Houbraken and Samson, (2011) some species of *Trichocomaceae* are important to both industry and medicine. Among the cellulolytic fungi, *Trichoderma spp.* and *Aspergillus spp*. have been widely studied for their ability to secrete high levels of cellulose-degrading enzymes (Baldrian and Gabriel, 2003). *Aspergillus spp.* is the major agents of decomposition and decay and as such produce a broad range of enzymes, including cellulase.

Cellulase characteristics and production by *Aspergillus spp*. have been well documented in many literature that Microorganisms having the ability to degrade cellulosic compounds are of great importance from different biological and ecological point of view. The cellulose degrading ability of fungal and bacterial species has provided a broad platform for research in determining the physico-biochemical properties of these microorganisms as well as their use in different biotechnological processes (Petre *et al.,* 1999). Fungal species hold a promising position in producing antibiotics and utilizing cellulose as a carbon source and a lot of work has been done on it (Raudonene and Varnayte, 1997). By far the most extensively studied fungi are the soft-rot fungi such as *Trichoderma viride and Trichoderma reesei.*

MATERIALS AND METHODS

Materials used include: Potato Dextrose Agar media (PDA), Distilled Water, Petridishes, Autoclave, Thermometer, Centrifuge, Deep refrezerator, Incubator, pH-Meter, Weighing Balance, Bunsen burner, Wire loops, Test tubes and Racks, Universal containers, Erlenmeyer flask, and other essential laboratory apparatus.

Sample collection

E. crassipes Samples were collected from Laxmi Taal situated in middle of Jhansi city. These samples were carried out to laboratory and rinsed with water to remove adhere dirt. Then two plants of these samples were put in each sterilize polythene bag. All bags properly sealed and labeled. For each samples, pits had dug out upto 24 inches depth and put all these samples for different days. After some time all polythene bags with decomposed *Echhornia* Plants were removed from pits and sent to laboratory where they were kept at 4^0C.

After cooling at room temperature, about 20 ml of the media was poured into different sterile petridishes and then left undisturbed until the agar solidified. The plates were maintained at aseptic condition.

Isolation of fungal organisms

The samples from decomposed *Eichhornia* were suspended in 10 ml of sterilized distilled water, followed by serial dilution of each sample into four different test tube. A loopful of each sample from the diluents was streaked on the solidified PDA media plates and incubated at 37^{o}C for 72 hours for the fungal growth.

The pure cultures were identified by their morphology and colony characteristics and sub-cultured using standard method. The organisms were maintained on PDA petridishes and slants and stored at 4^{o}C. Colonies were observed by light microscope using 10 and 40X objective lens. Pure colonies were transferred to PDA plate and used for identification.

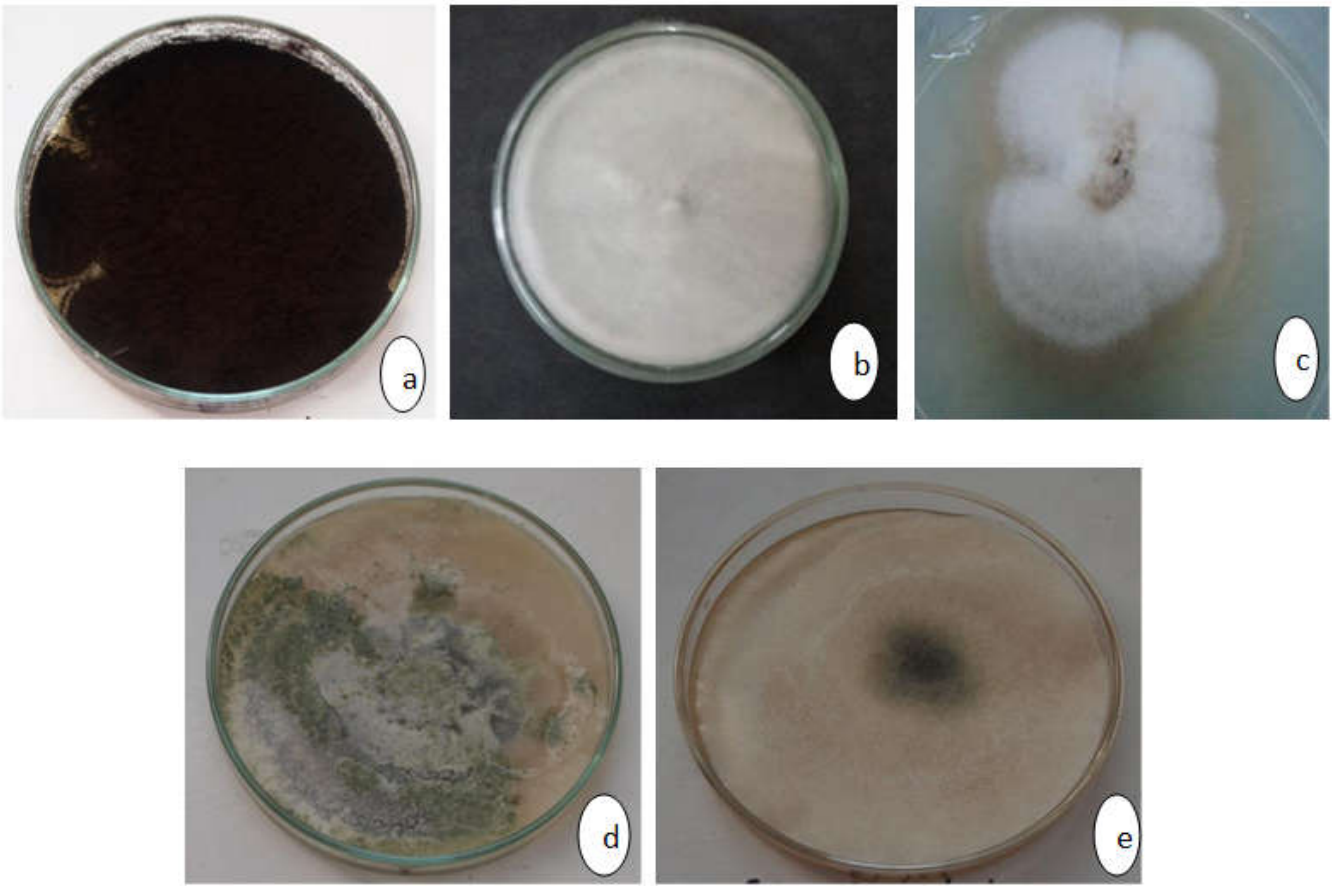

Plate-1. Fungi – (a) Aspergillus (b) Penicillium, Cladosporium and Mucor (c) Fusarium (d) Trichoderma (e) Rhizopus

Preparation of culture media

Potato Dextrose Agar (PDA) was prepared according to the manufacturer's specification. Potato was peeled, crushed and boiled with water and then mesh this bioled potato and filter. 20g of agar media powder and 15g dextrose were dissolved in distilled water and make up to 1000 ml media by adding distilled water in Erlenmeyer flask. The flask was covered with cotton-plug and foil and sterilized by autoclaving at 15lb/in^2 for 15 minutes.

Identification of fungi

Fungal culture was stained with Cotton blue and lacto phenol and covered with glass cover slip then observed in light microscope using low power (10X) and high power (40X) objective lens. Morphological characteristics of fungal culture were identified using laboratory manual for introductory mycology (Gilman, JC 1944, Onion *et al.* 1981, Smith *et al.* 1983).

Table-1. Microfungi growth on PDA

Fungi	Cellulose degrading fungi	7th days	15th days	30th days	45th days	60th days	90th days	120th days
Alternaria	----	-	+	+	-	+	++	+
Aspergillus niger	+++++	+	+	-	+	++	+	+++
Cladosporium	-----	-	-	-	+	+	-	+
Curvularia	----	-	-	+	-	+	+	-
Fusarium	++	-	+	++	+	+	++	+
Mucor	----	+	+	+	+	++	+	++
Nigrospora	----	-	-	-	+	-	+	+
Phoma	----	-	-	-	-	-	+	+
Pythium	-----	-	-	-	-	-	-	+
Rizhopus	+	-	+	+	++	+	+	++
Gliocladium	-----	-	-	-	-	+	+	+
Penicillium	++	-	+	+	+	++	+++	++
Trichoderma	+++++	+	+	+	+	++	++	+++
Helminthosporium	------	-	-					+

Fungal screening

The isolated fungal cultures were screened for their ability to produce cellulase on selective media contained $NaNO_3$–2g, K_2HPO_4-1g, $MgSO_4.7H_2O$–0.5g, KCl–0.5g, carboxy methyl cellulose sodium salt– 2g, Agar agar- 17g and distilled water- 1000 ml. pH of the medium was adjusted to 5.0. After autoclaving at 121°C and 15lb/in^2 pressure, the medium was poured into Petri plates and allowed to solidify then inoculated with fungal culture. The plates were incubated at room temperature for five days to allow fungal growth. After incubation, 10ml of 1% Congo red staining solution was added to each plate and was shaken for 15min. The Congo red staining solution was discarded and added 10ml of 1N NaCl then again shaken to de-stain plate for 15min. Finally 1N NaCl was discarded and the stained plates were analyzed by observing the formation of clear zone around the fungal colonies. The high zone of clearance showing fungal isolates was used for high cellulose production.

RESULTS AND DISCCUSION

In our result findings of *Aspregillus, Penicillium sp, Trichoderma sp, Mucor, Alternaria Cladosporium, Curvularia, Fusarium, Mucor, Nigrospora, Phoma ,Pythium, Rhizopus, Gliocladium and Helminthosporium* were found. *Aspergillus niger, Trichoderma, Penicllium Mucor and Rhizopus* were the dominant species, they show vigorous growth and were found in large numbers (Table-1). Morphologically, *Apergillus niger* have black colour colony with conidial production and *Penicillium* have Initially white and fluffy, later produced pigmented spores turn into bluish green on potato dextrose media. *Mucor* shown white cottony growth on PDA, nonseptate hyphae, having sporangiphore , sporangia and spores. *Trichoderma* was transparent at first on PDA, after seven days of incubation at 25-28°C, green colour tufts conidia form. From above isolates cellulose degrading microfungi were identified by subculturing on PDA containing 1% CMC on the basis of morphological characterstics and their colonies growth. Clear zones surrounded the colonies with zone diameter measuring 1.2 to 4.5 cm. On the basis of morphological characteristics and clear zone the isolates were identified as *A. niger. penicillium spp*, *Trichoderma spp, Fusarium spp and Rhizopus* but *Aspergillus* and *Trichoderma* were more potentially cellulose degrading microfungi deu to more clear zone diameter than other microfungi.

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