EVALUATION OF CELLULASE PRODUCING FUNGI ISOLATED FROM AGRICULTURAL WASTE

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Abstract - Cellulose is the highest amount of carbohydrate available in the plant biomass. Due to the increasing demand for starch as a source of glucose, there is a need to search alternative source of starch for glucose. Cellulose is an abundant and the cheap source of glucose in nature; hence there is huge demand for cellulose degrading enzymes. The present work aimed at screening and evaluating the cellulolytic fungi from the agricultural waste. In the present study we have collected agricultural waste samples from the sugarcane fields randomly. Then screened the samples for the presence of cellulose-enzyme producing fungi and performed the fungal enzyme production and efficacy of produced enzyme activity on the cellulose degradation. We isolated and identified five types of fungal species that are produced cellulase enzyme significantly. The results showed that cellulase production and the enzyme activity of Aspergillus species were higher than for Rhizopus, Penicillium, Actinomycetesand Candida species.

Key Words - Cellulase, agricultural waste, Fungi

I. INTRODUCTION

From studies, it has been found that cellulose is one of the most abundant component of plant biomass, although cellulose is produced by some animals for example tunicates and few bacteria, It is has been shown to occur almost in nature exclusively in plant cell walls (Lynd et al, 2002). Lasting renewal of plant biomass through the process of photosynthesis ensures an inexhaustible supply of such material. Any process that can proficiently and economically convert cellulolytic material to glucose is of industrial significance (Walsh, 2002). Cellulases have many industrial applications. When glucose is obtained from cellulosic substrate it can be used as substrate for subsequent fermentation or other processes which can produce useful end products such as butanol, ethanol, amino acid, methane, protein, etc. (Walsh, 2002). The importance of cellulose as source of energy that is renewable has made its hydrolysis the focus of many researches and interest of industrial processes (Bhat, 2000). Over the years, many organisms, in particular fungi, and enzymes that possess cellulose-degrading have been isolated and studied (Bhat and Bhat, 1997; Nowak et al, 2005). Many fungal organisms are also produce cellulose enzymes naturally. In the present study we collected agricultural wastes from local/rural areas in and around Tirupati, Andhra Pradesh, India and tested for the presence and evaluation of cellulose producing fungi from these samples.

II. MATERIALS & METHODS:

Collection of Samples & Isolation of fungal strains:

The soil sample was collected from the Agricultural areas of Tirupati, Andhra Pradesh, India from a depth of 1-15 inches from the top soil and sieved through a 2 mm sieve. The samples were dispensed into sterile bags and were carried to the laboratory. Soil enrichment analyses were done by adding 1g of cellulose to the sample. For the isolation process, the method of plate dilution was used. One gram (1g) of collected soil was placed in 10 ml of sterile water and shaken for proper mixing at room temperature. Then, 100 µl samples were inoculated onto the sterilized potato dextrose agar plates (25% potato & 1.5% agar w/v) under aseptic conditions. Inoculated plates were incubated at 28°C for 4 to 8 days. The Mandel's enriched medium was the selective medium used at pH 5 to get the desired fungi.

Screening of fungal isolates for cellulolytic activity

CMCase assay was used for screening (Mendels et al, 1976). This shows that out of six fungal isolates, five isolates were produced cellulase. Most of the producers were Aspergillus sp. followed by Penicillium sp., Rhizopus sp., Actinomycetes sp. Candia sp. showed no cellullytic activity (Table 1).

Measurement of cellulolytic activity

Endoglucanase (CMCase) assay was carried out for fungal isolates for their ability to degrade crystalline carboxymethylcellulose (Table -1).

Effect of temperature on enzyme production

Effect of temperature on enzyme production of different isolates was observed. Some Aspergillus sp. isolates showed high degree of production at 370C, while other species was shown high production at 300 C. Isolated Aspergillus cultures were grown at different temperatures and found the above results.

Effect of pH on enzyme activity

Maximum fungal isolates activities were found at pH values ranging from 4 to 4.8. The fungal isolates were tested at different pH ranges for their activity. Then it

was found that the enzyme activity was maximum between 4 to 4.8 of pH medium. (Kiman Siles, et al. 2017).

Induction of enzyme

Enzyme activity was induced 10 fold when Aspergillus niger was cultured on synthetic media with the sole carbon source as cellulose. Low level of enzyme activity was seen when fungus was grown in glucose containing medium as the sole carbon source. Previous work shows that cellulases production is strongly regulated. In many cellulose degraders, readily utilizable carbon sources generally suppress cellulase production by a mechanism thought to be similar to catabolite repression (Lederberg, 1992).

III. RESULTS

Isolation and identification of fungi

In total, six isolates of fungi were isolated and identified up to genus level. Based on the colony morphological characteristics (stained with Lacto phenol cotton blue) observed under microscopes, the following fungal isolates were identified. These includes: Aspergillus niger, Penicillium sp., Rhizopus sp., Candida sp., and Actinomycetes sp.

IV. DISCUSSION

As world is in high demand for alternative energy sources such as bioenergy the most reliable and abundant natural source is cellulose. Cellulose is the cheapest and readily available plant material. It has been found as the major component of biomass energy (Scott et al, 1987).

Because majority of vegetation added to soil is cellulose therefore, the decomposition of cellulose has as important significance in the biological cycle of carbon (Lederberg, 1992). Decomposition of the celluloses are naturally occurs in the soil by the action of certain cellulases that are produced by microbes. Industrially, these enzymes have found new use in the production and processing of food, chemicals, and manufactured goods such as rayon, paper, etc. Cellulose has also been used for the extraction of valuable components from plants and for the enhancement of nutrition values of animal feed (Wiseman, 1995). In general, fungi are known for their decomposition ability of organic matter and of cellulosic substrate in particular as has been reported by Lynd et al, (2002). Among the six fungal strains isolated, only five types of species, are most extensively studied as cellulase producers Fungi are well known for their decomposition ability of organic matter in general and in particular as cellulosic substrate as has been (Lynd et al, 2002). Among the six fungal strains isolated, only five types of species are most extensively studied as cellulase producers. This could be because of the possibility of rapidly grown fungi like Aspergillursflavus, Aspergillusniger,Rhizopus sp., Penicillum sp.andActinomycetes species. Sari et al, (2017) reported that among cellulose producing fungi isolates Aspergillusflavus showed more cellulose activity. The present study revealed that the cellulase activity of Aspergillus species were found comparatively higher cellulase activity than for Rhizopus sp., Penicillum sp., Actinomycetes sp. Santos et al. (2016) also reported that considerable number of cellulose has been produced by fungi such as Aspergillusand Rhizopus species. Maximum enzyme production by Aspergillus species at 37°C was previously reported by Sone et al., (1999) &(Kiman Siles, et al. 2017).

The result of this study partly confirms the report that higher enzyme yield was found at 37°C than at 30 °C. Higher enzyme yield at 30°C was reported for some Aspergillus strains by Philippidis (1994). In this study, we isolated six types of fungi; out of five fungi showed cellulolitic activity. Among the five types Aspergillus sp. and Rhizopus sp. Showed high enzymatic activity than other isolates. The further studies of the above isolates can lead the effective strains isolation and utilization at commercial scale.

Table: 1. Enzyme activity on cellulose (CMC) by fungal isolates:

S.No.	Isolated Fungi	Enzyme activity (U/ml)
1	*Aspergillus niger*	2.022
2	*Aspergillus sp.*	1.368
3	*Penicillium sp.*	0.043
4	*Rhizopus sp.*	0.141
5	*Actinomycetes sp.*	0.089

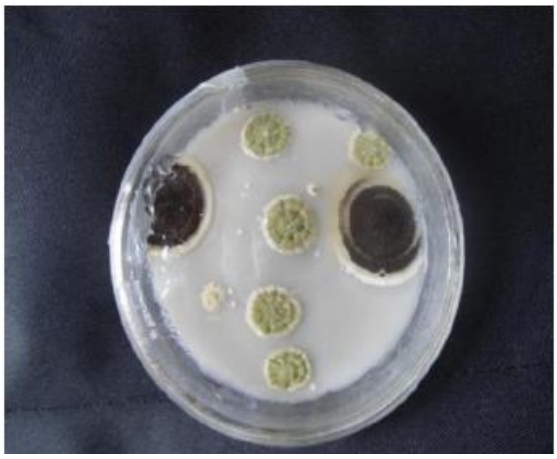


Fig-1 Isolated fungal colony



Fig-2 Isolated Fungal colony



Fig-3. Isolated fungal colony



Fig-4.Isolated fungal colony

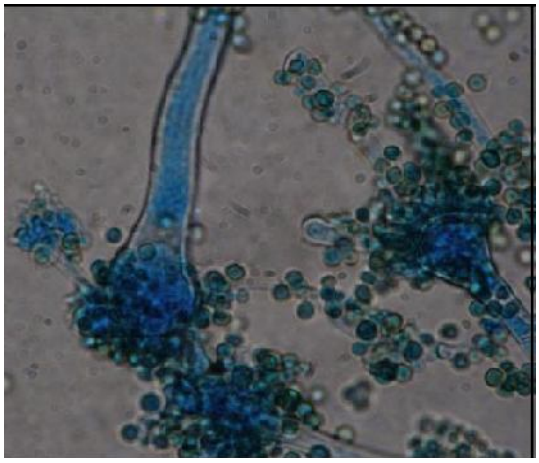


Fig: 5 Microscopic observations of stained fungal isolates

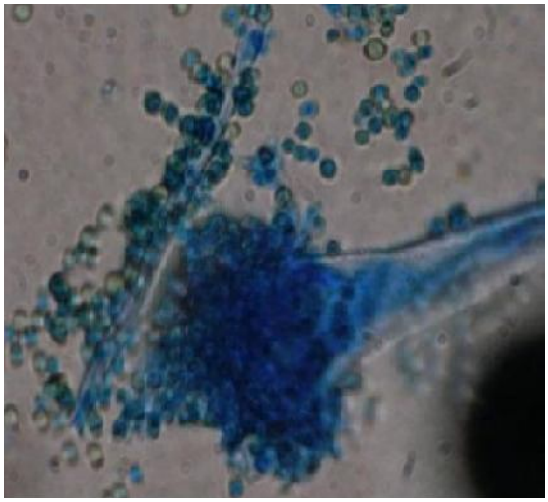


Fig:6. Microscopic observations of stained fungal isolates

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