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Research Article

CO-CULTURING OF SOIL FUNGI FOR AGRICULTURAL WASTE MANAGEMENT

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ABSTRACT

The most abundant organic compound and a prominent carbonaceous consistuent of higher plants and probably is cellulose. Because a large part of the vegetation added to soil is cellulose, the decomposition of the carbohydrate has a special significance in the biological cycle of carbon. Cellulose is a polysaccharide composed of glucose units in a long linear chain linked together by β-1,4 glycosidic bonds. Degradation of cellulose is brought about by fungi, bacteria and actinomycetes by the secretion of extracellular enzyme, cellulase. It is a complex enzyme composed of three components, endoglucanase, exoglucanase and a β-glucosidase. Cellulase has a wide application in textile industry, in laundry detergents, in pulp and paper industry, in pharmaceutical, commercial food processing in coffee, in fermentation of biomass into bio fuels, and also used as a treatment for phytobezoars, a form of cellulose bezoar found in human stomach. Sugarcane baggase and Paddy straw are a complex substrates obtained from processing of sugarcane and drying of paddy. Due to their abundant availability, it serve as an ideal substrate for microbial processes for the production of value added products. The present study was conducted to isolate and identify *Aspergillus* sp and *Penicillium* sp from the soil and to estimate cellulase enzyme production by the isolates in single and in co-culture on cellulosic agricultural waste for cellulose production.

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INTRODUCTION

Cellulosic biomass constitutes the most abundant organic molecules on earth and is continually replenished by carbon dioxide fixation via photosynthesis. All cellulosic materials, including the agro-industrial wastes can be converted into commercially important products such as ethanol, methane, glucose syrups and single cell proteins. The products of degradation constitute both nutrients for growth and regulators of the production of ligno-cellulolytic enzymes**.** Bioconversion, particularly enzymatic hydrolysis, of these cellulosic materials into simple sugars, has been a subject of intensive research. The development of an industrial process for cellulose bioconversion would help alleviate shortages in food and animal feeds and also reduce the problems of urban waste disposal and overdependence on fossil fuels. Successful utilization of these renewable resources is dependent on the development of an economically viable process which would include the production of cellulases required for the enzymatic hydrolysis of cellulosic materials (Walton *et.al*., 1997). Cellulose is commonly degraded by an enzyme called cellulase. Cellulase, a group of hydrolytic enzymes which hydrolyze the glycosidic bonds of native cellulose and related cellulooligosaccharides, it is a key enzyme of potential use for industrial saccharification of cellulosic materials into simple sugars. Cellulases form an important part of food, animal and textile industry, they are also used in waster resource recycle management and anti-pollution treatment (Tarek and Nagwa,2007). The enzyme cellulase was produced by several microorganisms, mainly by bacteria and fungi. Although a large numbers of microorganisms are capable of degrading cellulose, only few of these produce significant qualities of cell free enzymes capable of completely hydrolyzing crystalline cellulose invitro (Akiba and Kimura, 1995).

Most of the products in industrial biotechnology today are formed using processes involving a single microbial strain because sterile cultivation enables an easy way of controlling microbial milieu, growth and product formation. On the other

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hand, there are many instances where the utilization for cocultures appears to be advantageous over a single microorganism because of the potential for synergistic utilization of the metabolic pathways of all involved strains in a coculture situation (Belenguer *et.al*.,2006). Anaerobic or aerobic incubation of different specified microbial strains under aseptic conditions is known as co-culture. In co-cultures, degradation and metabolization of substrates occur by the combined metabolic activity of the known microbial strains under aseptic conditions. Co-cultivation may result in increased yields, a reduction of process costs because of a cheaper (sometimes even unsterile) substrates and control of product quality. In some cases, production of substances normally not formed by pure cultures can be observed through the induction of appropriate genes in cocultivation processes. Coculture fermentation may have a great impact on the development of biofuels, bioenergy and biobased products (Taniguchi *et.al*.,2004). Cocultures of different microorganisms may be also advantageous for the production of enzymes. Increased production of cellulases by a coculture of two moulds *Aspergillus ellipticus* and *Aspergillus fumigates* was described by Gupte and Madamwar,(1997). Also, the cocultivation of *Aspergillus niger* and *Trichoderma reesei* increased cellulase production significantly (Ahamed and Vermette,1998).

MATERIALS AND METHODS

Isolation of Aspergillus sp and Penicillium sp from soil

Aspergillus sp and *Penicillium* sp were isolated from soil sample by serial dilution technique. Plates were Incubated at room temperature for 7 days and identified based on their morphological characteristics and microscopic observation using the lab manual by Nagamani,(2006).Pure cultured and maintained on Czapek's Dox Media (Aneja,2007).

Study for synergism between Aspergillus sp and Penicillium sp

Aspergillus sp and *Penicillium* sp were streaked on Czapek-Dox Agar media. Incubated at room temperature for 7 days. Observed for synergism (Dubey and Maheshwari, 2002).

Production of CMCase enzyme by the isolates

Individual and co-cultures were inoculated on to modified Czapek's mineral salt broth medium.Incubated at room temperature on rotary shaker for 5 days. Centrifuged at 5,000rpm for 10 minutes.Supernatant was collected and CMCase activity estimated (Modified Method of Aneja, 2007).

Estimation of CMCase enzyme activity

Endoglucanase activity

0.45ml of 1% CMC solution was taken.0.05ml of enzyme extract was added. Incubated at 55^0c for 15mints.0.5ml of DNS reagent was added. Incubated in boiling water bath for 5mints.1ml of potassium sodium tartarate solution was added and cooled.5ml of distilled water was added. Absorbance measured at 540nm.Standard graph of glucose was plotted and Endoglucanase activity was determined using the standard graph of glucose (Sadasivam and Manickam, 2008)

Exoglucanase activity

32mg of dry whatmann No.1 filter paper was taken.0.5ml of enzyme extract was added. Incubated at 50^0c for 1hr.0.5ml of DNS reagent was added. Incubated in boiling water bath for 5mints.1ml of potassium sodium tartarate solution was added.3ml of distilled water was added. Absorbance was measured at 540nm.Standard graph of glucose was plotted and Exoglucanase activity was determined using the standard graph of glucose (Sadasivam and Manickam, 2008)

SSF of Aspergillus sp and Penicillium sp on paddy straw and sugarcane baggase

1g of paddy straw was taken. Moisture percentage was adjusted to 70% and pH to 4.5 using 0.1M acetate buffer. Autoclaved at 121^0c for 45mints.5ml of spore suspension was inoculated. Incubated at 37^0c.Samples were withdrawn periodically at an interval of 2days (2, 4, 6,8,10 days).30ml of 0.1M acetate buffer of pH 4.5 was added. The mixture was blended using hand blender. Centrifuged at 3,000rpm at 4^0c for 20mints. Supernatant was assayed for CMCase activity. (Gutierrez-Correa and Tengerdy, 2000)

RESULTS AND DISCUSSION

The objective of the present study was to isolate and identify *Aspergillus* sp and *Penicillium* sp from soil. The co-culture of two cellulolytic organisms (*Aspergillus* sp and *Penicillium* sp) was studied to see their synergestic cellulose degrading activity on a soild substrate i.e., sugarcane baggase and paddy straw by soild-substrate fermentation.

Aspergillus sp and *Penicillium* sp were isolated from the soil and were identified based on morphological characteristics and microscopic observation.

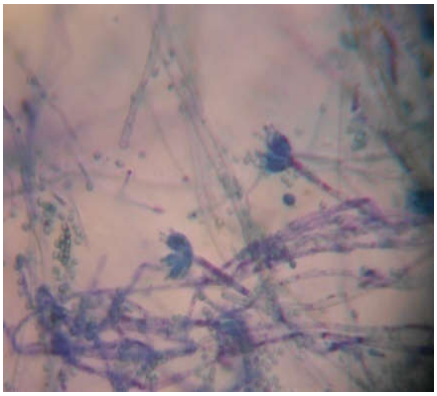

Fig 1 *Penicillium* sp

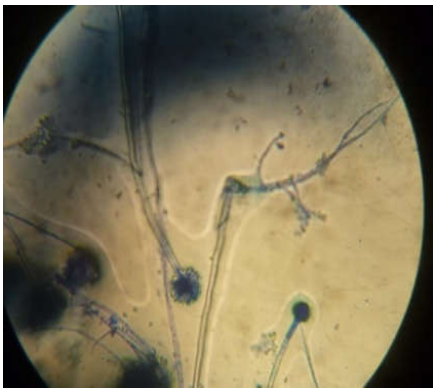

Fig 2 *Aspergillus* sp.

Aspergillus sp and *Penicillium* sp were studied for synergistic activity. It was found that both the culture grow well together and showed positive result for synergism as shown.

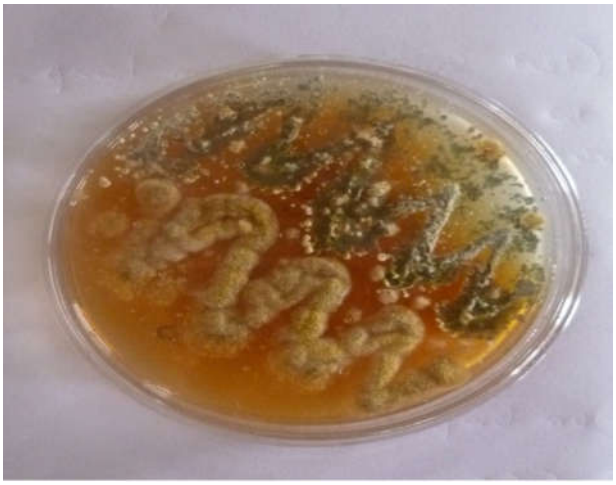

Fig 3 Synergism between *Aspergillus* sp and *Penicillium* sp

Endoglucanase activity and exoglucanase activity was estimated using standard graph of glucose. The isolates in pure culture and co-culture were inoculated on modified Czpake's Mineral Salt Broth Medium and studied for Endoglucanase activity. The results are as shown in Table 1. One cellulase unit is the mg glucose released per minute per mg protein. It was observed that Endoglucanase and exoglucanase activity was high in co-culture compared to *Aspergillus* sp and *Penicillium* sp. Exoglucanase Activity (7.9±0.03 IU/ml) was found to be higher in Co-culture as compared to Endoglucanase activity (5.8±0 IU/ml). 12 days of incubation days was found to be ideal for both Endoglucanase activity and exoglucanase activity at 25^0C. According to Singh A., *et.al*.,(2009), maximum cellulase activity was observed on 5^{th} day of incubation at 30^0c.

Table 1 Enzyme Production by Isolates in Modified Czpake's Mineral Salt Broth Medium

Substrate	Incubation days	Endoglucanase Activity IU/ml	Exoglucanase Activity IU/ml
Penicillium sp	0	0. 9±0.01^{b}	0.15±0.01^{a}
	3	2. 3±0.01^{e}	1.89±0.01^{o}
	6	3. 7±0.01^{g}	2.30±0^{n}
	9	4. 6±0.02^{j}	3.2±0^{l}
	12	4. 3±0.02^{m}	2.92±0^{k}
	15	3.43±0.03^{o}	2.98±0^{h}
	18	1.21±0.01^{q}	0.89±0^{f}
Aspergillus sp	0	0.7±0.01^{f}	0.2±0.01 ^{b}
	3	1.6±0.01^{h}	1.65±0.01^{p}
	6	2.17±0.01^{k}	1.89±0.01^{m}
	9	3.72±0.08^{n}	2.75±0.05^{j}
	12	4.20±0.025^{r}	2.86±0.04^{g}
	15	3.79±0.03^{s}	1.95±0.03^{f}
	18	2.28±0.02^{t}	0.06±0.04^{d}
Co-Culture	0	0.15±0.0^{a}	0.3±0.04^{c}
	3	1.39±0.03^{c}	1.43±0.01^{r}
	6	2.28±0.01^{d}	3.16±0.02^{q}
	9	3.21±0.01^{i}	6.62±0.02^{p}
	12	5.8±0^{j}	7.9±0.03^{m}
	15	3.6±0^{l}	6.14±0.02^{i}
	18	2.9±0^{p}	4.25±0.02^{e}

P.N: Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at P≤0.05 according to Duncan's multiple range test.

Solid- substrate fermentation of *Aspergillus* sp, *Penicillium* sp and co-culture was carried out using paddy straw and sugarcane baggase as a substrate. The paddy straw optimized to 70% moisture, pH 4.5 and incubated with the isolates for 180 days at 25^0c and assayed for endoglucanase activity and exoglucanase activity. The results are as shown in Table 2. It was observed that Endoglucanase and exoglucanase activity was high in co-culture compared to *Aspergillus* sp and *Penicillium* sp.inoculated in paddy straw and sugarcane baggase as substrates respectively. When sugarcane bagasse was used as substrate both endoglucanase Activity (14.27±0.04 IU/ml) and exoglucanase activity(13.94±1.63 IU/ml) was found to be higher in Co-culture as compared to *Aspergillus* sp, *Penicillium* sp.respectively. When paddy straw was used as substrate, endoglucanase Activity (12.46±0.14 IU/ml) and exoglucanase activity (13.22±0.01 IU/ml) was found to be higher in Co-culture as compared to *Aspergillus* sp, *Penicillium* sp.respectively.90 days of incubation period ws found to be optimum for cellulase production.

Table 2 Enzyme production by isolates during SSF of substrates

Substrate	Incubation days	Endoglucanase Activity IU/ml		Exoglucanase Activity IU/ml	
		Sugarcane bagasse	Paddy straw	Sugarcane bagasse	Paddy straw
Penicillium sp	0	0.36±0.04^{p}	0.78±0.08k	0.93±0.47^{b}	0.37±0.01^{a}
	30	3.78±0.03^{m}	1.13±0.07^{i}	3.35±0.57^{c}	3.63±0.01^{c}
	60	6.2±0.20^{j}	3.5±0.55^{h}	6.64±0.83^{f}	6.76±0.01^{a}
	90	9.4±0.40i	9.61±0.51^{f}	11.86±0.94^{g}	10.83±0.02^{g}
	120	12.35±0.25^{h}	10.55±0.55^{e}	10. 85±1.19^{h}	8.80±0.01^{i}
	150	8.35±0.25^{e}	10.02±0.40^{e}	5.85±1.4^{6j}	4.94±0.01^{l}
	180	7.5±0.30^{b}	9.1±0.10^{d}	2.55±1.81^{l}	1.32± 0.02^{m}
Aspergillus sp	0	0. 4±0.05^{l}	0.9±0.10^{i}	0.8±0.35^{a}	0. 8±0.01^{a}
	30	3.64±0.50^{j}	3.2±0.20^{j}	2.21±0.61^{d}	3.70±0.02^{c}
	60	5.45±0.55^{i}	6.3±0.20h^{-i}	5.88±1.45^{j}	6.75±0.005^{d}
	90	11±0.50^{h}	10.26±0.47^{g}	9.76±2.38^{o}	8.88±0.01^{f}
	120	8.7±0.50^{f}	9.1±0.10^{f}	7.74±2.88^{q}	7.96±0.01^{h}
	150	5±0.50^{d}	7.22±0.09^{d}	6.09±3.05^{r}	5.37±0.01^{j}
	180	3.56±0.49^{c}	7.11±0.09^{c}	5.14±3.46^{s}	4.92±0.02^{k}
Co-Culture	0	0. 7±0.13^{n}	0.99±0.10^{l}	0. 8±0.45^{b}	0.5±0.01^{a}
	30	2. 9±0.31^{p}	4.6±0.30^{f}	3.37±0.69^{e}	5.68±0.01^{b}
	60	6.5±0.31^{o}	8.3±0.20^{e}	7.62±1.32^{i}	9.79±0.01^{d}
	90	14.27±0.04^{k}	12.46±0.14^{d}	13.94±1.63^{k}	13.22±0.01^{e}
	120	10.30±0.005^{i}	9.68±0.03^{c}	8.98±1.99^{m}	10.33±0.01^{f}
	150	7.36±0.50^{g}	6.11±0.10^{b}	6.66±2.34^{n}	7.98±0.01^{g}
	180	4. 6±0.39^{a}	3.81±0.09^{a}	3.21±2.61^{p}	4.52±0.01^{i}

P.N: Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at P≤0.05 according to Duncan's multiple range test.

According to Haltrich *et.al*.,(1999), the highest enzyme activity was shown in co-culture of *Aspergillus niger* and *Penicillium chrysogenum* and it raised to 3.56 IU/ml on 60 days of incubation and it was very high as compared with another co-culture media. Jayant *et.al*.,(2011), reported that *Aspergillus* MAM-F23 gave the highest CMCase (309 U/ml) on paddy straw. The enzyme activity was being maximal at pH 5. The cellulase was stable over a wide range of pH values. Initial pH has a direct effect on the uptake of mineral nutrients, which are present in the fermentation medium. Effect of different pH (3.0 - 6.0) of fermentation medium on the enzymes production was investigated Henrissat *et.al*., (2000) with simultaneous co-culturing of *A. niger* and *T. viride.* Mrudula *et al*.,(2011) have shown that Sugarcane bagasse, tea production waste, coconut coir pith, rice husk, wheat bran, rice bran etc., have been

employed for production of cellulase using a variety of microorganisms such as *Trichoderma, Aspergillus, Penicillium, Botrytis, Neurospora* etc.,

CONCLUSION

Co-culturing of microorganisms is found to be a best method for the production of cellulase enzyme which results in increased yield. From the present study it can be concluded that, *Aspergillus* sp and *Penicillium* sp can be used as a source of industrially important cellulase enzyme. The Co-culturing of *Aspergillus* sp and *Penicillium* sp showed maximum exoglucanase activity on both paddy straw and sugarcane baggase. The solid waste of sugar, paper and industry using baggase, paddy straw, paper waste and cotton waste can be fermented by *Aspergillus* sp and *Penicillium* sp by solid-substrate fermentation. The co-culturing of these fungi can thus be used efficiently to manage various agricultural waste thereby controlling pollution.

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