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# Efficacy of *Aspergillus fumigatus* in cellulase enzyme complex production with bagasse as substrate

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## SUMMARY

Concentration (0.1, 0.2, 0.3 and 0.4 per cent) of carbon sources on the 5th, 7th and 9th days of incubation exhibited by *Aspergillus fumigatus* in the presence of bagasse as substrate. Results of the study revealed that among the carbon sources the intracellular exoglucanase and β glucosidase activity were registered at a higher level of 1.913$Uml^{-1}$and 0.689 $Uml^{-1}$ in 0.4 per cent dextrose. The endoglucanase activity was found to be expressed maximally in 0.4 percent maltose (2.470$Uml^{-1}$) than the control. Extracellular exoglucanase and β -glucosidase were significantly recorded at maximum level of 2.057 $Uml^{-1}$ and 0.403 $Uml^{-1}$ in 0.4 per cent fructose as carbon source. The endoglucanase activity was very much pronounced (1.620$Uml^{-1}$) in 0.4 per cent dextrose when compared to the control.

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**Key words :** Extracellular, Intracellular, Exoglucanase, Endoglucanase and β-glucosidase

Plant cell wall polysaccharides are the most abundant organic compounds found in nature. They make up 90 per cent of the plant cell wall and can be divided into three groups namely cellulose, hemi-cellulose and pectin. Cellulose is indigestible by humans because humans do not produce the enzyme cellulase. Cellulase is produced by grazing animals such as cows (with the aid of the beneficial bacteria that reside in the animals digestive tract), and is the reason why they can get nutrition from plants such as grasses. Cellulose consists of linear β-1, 4-linked d-glucopyranase chains that are condensed by hydrogen bonds into crystalline structures, called micro-fibrils. These micro-fibrils consist of upto 250 glucose chains and are linked by hemicellulose. In addition to this crystalline structure, cellulose contains non-crystalline (amorphous) regions within the micro fibrils. Cellulase refers to a family of enzymes which act in concert to hydrolyze cellulose. Cellulases are widely distributed throughout the biosphere and are manifested mostly in fungal and microbial organisms. Cellulase enzyme complex converts crystalline, amorphous and chemically derived celluloses qualitatively to glucose. Cellulolytic enzymes are generally formed as multi enzyme systems and have classified into three major groups as exoglucanase, endoglucanase and β –glucosidase.

The synergistic action of these three enzymes is required for complete degradation of cellulose. The extracellular production of microbial cellulases depends on a number of factors such as inoculum size, carbon source, pH, temperature, presence of inducers or inhibitors, medium additives, batch size, aeration and growth time (Bisaria and Ghose 1981, Saddler *et al.,* 1987). Many microorganisms, mostly fungi, degrade cellulosic and hemicellulosic materials and produce a complete set of cellulases for the hydrolysis of cellulose and hemicellulose to respective sugars (Coughlam, 1985). Several studies were carried out to produce cellulolytic enzymes from biowaste degradation process by many organisms including fungi such as *Trichoderma, Aspergillus* spp.*, Penicillium* (Lakshmikant and Mathur, 1990). Since the production of cellulase enzyme is a major process and economically viable, much work has been done on the production of cellulase from lignocellulosics and major attention has been given to use baggase as substrate.

With this background, the present investigation was carried out to analyze the efficacy of *Aspergillus fumigatus* in the production of cellulase enzyme complex like exo- β- 1,4 glucanase ($C_1$-cellulase), endo- β-1,4 glucanase ($C_x$-cellulase) and β-glucosidase (filter paper activity) at extracellular and intracellular level at different

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concentrations of carbon sources in the presence of the substrate (baggase).

## MATERIALS AND METHODS

*Aspergillus fumigatus* bought from Institute of Microbial Technology, Chandigarh, India was cultured on a PDA medium (Riker and Riker, 1936) on the Petriplates and agar slants were also maintained. Fifty ml of Czpek-Dox liquid medium (Raper and Thom, 1949) was dispensed in 250ml Erlenmeyer flasks and sterilized at 1 atm for 15 minutes. After cooling, one ml of streptomycin sulphate (10,000 ppm) was added. The pH of the medium was maintained at 6.5 after sterilization. *A. fumigatus* grown on PDA medium for five days in Petridishes was taken with the help of sterilized cork borer (10mm dia) and were inoculated to the liquid medium. The flasks were incubated for 5-7 days. The cellulolytic fungi, *A. fumigatus* was assayed for its cellulase enzyme complex activity (Sadasivam and Manikam, 1996) like exo,(C1), endo β 1,4 glucanases($C_X$) and filter paper (β-Glucosidase) activity in the culture filtrate of the fungi (extracellular) in the mycelium (intracellular) and with the substrate (bagasse)

The mycelium was filtered through Whatman filter paper using a Buchner funnel under suction and the clear filtrate was used as the source of enzyme (extracellular enzyme). The fungal mycelium was washed with distilled water twice. A quantity of one g of the washed mycelial mat was macerated in five ml of sodium acetate buffer, (pH 5.2) in a pre chilled motar and pestle with a pinch of acid washed sand. The homogenate was centrifuged in a refrigerated centrifuge at 10,000 x g for 15 minutes. The supernatant served as enzyme source.

## RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been presented under following heads :

### Exo β-1,4 glucanase activity ($C_1$ -cellulase):

Extracellular exoglucanase activity in *A. fumigatus* on the 9th day of incubation in bagasse as substrate was very much pronounced (2.057$Uml^{-1}$) in 0.4 per cent fructose than the intracellular exoglucanase activity shown in dextrose (1.913$Uml^{-1}$). The least intracellular value of 0.252$Uml^{-1}$was recorded by 0.4 per cent fructose and least extracellular activity of 0.280$Uml^{-1}$ was shown in 0.4 per cent sucrose as carbon source. The present finding is at par with result of Madamwar and Patel, 1992. They obtained highest enzyme activities by semi-solid state fermentation with *Aspergillus niger*, *Trichoderma reesei* and with co-culture of *T. reesei* and *A. niger* on different cellulosic materials on the 5th day in the untreated bagasse. The activity of exoglucanase were 8.2, 11.8, 16.2 U/ml, endoglucanase activity were 16.6, 19.6, 25.3 U/ml and β-glucosidase activities were 6.8, 3.2, 10.3 U/ml, respectively

### Endo β-1,4 glucanase activity (Cx -cellulase):

The intracellular endoglucanase activity of *A. fumigatus* on the 9th day of incubation at 0.4 per cent carbon source was expressed at a higher level as 2.470$Uml^{-1}$ in maltose when compared to extracellular endoglucanase activity of 1.620$Uml^{-1}$ shown in dextrose. The least intra and extracellular enzyme activities were expressed at a lower level of 0.552$Uml^{-1}$ and 0.052 $Uml^{-1}$ in 0.3 per cent sucrose as carbon source. The

**Table 1 : Exoglucanase activity in the presence of substrate**

| Carbon sources | Control | | | 0.1 Conc. | | | 0.2 Conc. | | | 0.3 Conc. | | | 0.4 Conc. | | | C.D. P<0.01 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | |
| **Extracellular** | | | | | | | | | | | | | | | | |
| Maltose | 0.058 | 0.058 | 0.345 | 0.221 | 0.249 | 0.363 | 0.142 | 0.145 | 0.943 | 0.275 | 0.338 | 0.695 | 0.091 | 0.132 | 0.914 | 0.004 |
| Dextrose | 0.058 | 0.058 | 0.345 | 0.092 | 0.113 | 0.573 | 0.326 | 0.399 | 1.147 | 0.299 | 0.337 | 0.322 | 0.063 | 0.331 | 1.048 | 0.004 |
| Sucrose | 0.058 | 0.058 | 0.345 | 0.146 | 0.161 | 0.440 | 0.144 | 0.154 | 0.790 | 0.142 | 0.154 | 0.484 | 0.222 | 0.332 | 0.280 | 0.005 |
| Fructose | 0.058 | 0.058 | 0.345 | 0.70 | 0.117 | 0.213 | 0.050 | 0.072 | 0.085 | 0.078 | 0.106 | 0.425 | 0.433 | 0.827 | 2.057 | 0.014 |
| **Intracellular** | | | | | | | | | | | | | | | | |
| Maltose | 0.191 | 0.236 | 0.403 | 0.329 | 0.632 | 0.398 | 0.115 | 0.242 | 1.649 | 0.254 | 0.312 | 1.895 | 0.207 | 0.209 | 0.744 | 0.006 |
| Dextrose | 0.191 | 0.236 | 0.403 | 0.110 | 0.122 | 0.738 | 0.104 | 0.144 | 0.770 | 0.108 | 0.121 | 0.968 | 0.103 | 0.113 | 1.913 | 0.015 |
| Sucrose | 0.191 | 0.236 | 0.403 | 0.141 | 0.172 | 0.652 | 0.138 | 0.139 | 0.302 | 0.126 | 0.129 | 0.311 | 0.329 | 0.632 | 1.398 | 0.016 |
| Fructose | 0.191 | 0.236 | 0.403 | 0.051 | 0.071 | 0.141 | 0.088 | 0.088 | 0.249 | 0.062 | 0.138 | 0.125 | 0.251 | 0.228 | 0.252 | 0.004 |

*Enzyme activity expressed in $Uml^{-1}mg^{-1}$ enzyme protein
. One $Uml^{-1}$ = mg glucose formed $min^{-1}mg^{-1}$ enzyme protein

**Table 2 : Endoglucanase activity in the presence of substrate**

| Carbon sources | Control | | | 0.1 Conc. | | | 0.2 Conc. | | | 0.3 Conc. | | | 0.4 Conc. | | | C.D. P<0.01 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | |
| **Extracellular** | | | | | | | | | | | | | | | | |
| Maltose | 0.032 | 0.043 | 0.047 | 0.153 | 0.168 | 0.299 | 0.162 | 0.168 | 0.197 | 0.153 | 0.168 | 0.299 | 0.238 | 0.268 | 0.806 | 0.004 |
| Dextrose | 0.032 | 0.043 | 0.047 | 0.170 | 0.100 | 0.150 | 0.250 | 0.250 | 0.290 | 0.120 | 0.210 | 0.42 | 0.060 | 0.100 | 1.620 | 0.668 |
| Sucrose | 0.032 | 0.043 | 0.047 | 0.070 | 0.073 | 0.075 | 0.017 | 0.060 | 0.184 | 0.049 | 0.050 | 0.052 | 0.056 | 0.056 | 0.122 | 0.004 |
| Fructose | 0.032 | 0.043 | 0.047 | 0.025 | 0.068 | 0.070 | 0.064 | 0.088 | 0.090 | 0.082 | 0.087 | 0.107 | 0.248 | 0.249 | 0.295 | 0.066 |
| **Intracellular** | | | | | | | | | | | | | | | | |
| Maltose | 0.026 | 0.027 | 0.750 | 0.318 | 0.245 | 0.306 | 0.106 | 0.336 | 0.354 | 0.033 | 0.053 | 0.336 | 0.224 | 0.285 | 2.470 | 0.005 |
| Dextrose | 0.026 | 0.027 | 0.750 | 0.027 | 0.046 | 0.208 | 0.061 | 0.301 | 0.357 | 0.086 | 0.088 | 0.501 | 0.096 | 0.359 | 0.737 | 0.267 |
| Sucrose | 0.026 | 0.027 | 0.750 | 0.032 | 0.042 | 0.061 | 0.056 | 0.106 | 0.107 | 0.082 | 0.088 | 0.187 | 0.086 | 0.121 | 0.552 | 0.004 |
| Fructose | 0.026 | 0.027 | 0.750 | 0.053 | 0.102 | 0.125 | 0.051 | 0.057 | 0.071 | 0.029 | 0.029 | 0.141 | 0.322 | 0.591 | 1.129 | 0.004 |

*Enzyme activity expressed in $Uml^{-1}mg^{-1}$ enzyme protein

. One $Uml^{-1}$ = mg glucose formed $min^{-1}mg^{-1}$ enzyme protein

**Table 3 : Filter paper activity in the presence of substrate**

| Carbon sources | Control | | | 0.1 Conc. | | | 0.2 Conc. | | | 0.3 Conc. | | | 0.4 Conc. | | | C.D. P<0.01 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | 5 | 7 | 9 | |
| **Extracellular** | | | | | | | | | | | | | | | | |
| Maltose | 0.048 | 0.049 | 0.052 | 0.034 | 0.036 | 0.383 | 0.034 | 0.036 | 0.106 | 0.053 | 0.063 | 0.108 | 0.138 | 0.139 | 0.157 | 0.004 |
| Dextrose | 0.048 | 0.049 | 0.052 | 0.049 | 0.096 | 0.154 | 0.021 | 0.067 | 0.272 | 0.065 | 0.073 | 0.322 | 0.093 | 0.093 | 0.376 | 0.004 |
| Sucrose | 0.048 | 0.049 | 0.052 | 0.064 | 0.079 | 0.085 | 0.092 | 0.072 | 0.110 | 0.026 | 0.089 | 0.098 | 0.072 | 0.092 | 0.110 | 0.004 |
| Fructose | 0.048 | 0.049 | 0.052 | 0.023 | 0.072 | 0.115 | 0.045 | 0.099 | 0.116 | 0.024 | 0.038 | 0.278 | 0.204 | 0.301 | 0.403 | 0.010 |
| **Intracellular** | | | | | | | | | | | | | | | | |
| Maltose | 0.060 | 0.063 | 0.084 | 0.066 | 0.077 | 0.152 | 0.111 | 0.125 | 0.134 | 0.102 | 0.123 | 0.414 | 0.295 | 0.323 | 0.605 | 0.004 |
| Dextrose | 0.060 | 0.063 | 0.084 | 0.084 | 0.097 | 0.224 | 0.021 | 0.048 | 0.531 | 0.096 | 0.544 | 0.619 | 0.096 | 0.419 | 0.689 | 0.004 |
| Sucrose | 0.060 | 0.063 | 0.084 | 0.067 | 0.078 | 0.082 | 0.064 | 0.068 | 0.110 | 0.079 | 0.101 | 0.124 | 0.090 | 0.111 | 0.175 | 0.061 |
| Fructose | 0.060 | 0.063 | 0.084 | 0.008 | 0.010 | 0.101 | 0.043 | 0.043 | 0.072 | 0.056 | 0.057 | 0.073 | 0.111 | 0.130 | 0.186 | 0.005 |

*Enzyme activity expressed in $Uml^{-1}mg^{-1}$ enzyme protein

. One $Uml^{-1}$ = mg glucose formed $min^{-1}mg^{-1}$ enzyme protein

present finding is at par with the result of Maheswari *et al.* (1998). They found that wheat straw pre-treated with either alkali or steam or both together, inoculated with *Trichoderma reesei* enhanced carboxymethyl cellulase and filter paper cellulase activities by 52% and 74%, respectively in the treated substrate compared with the untreated one.

### β-Glucosidase (filter paper activity ):

Intracellular β- glucosidase activity of *A. fumigatus* on the 9th day of incubation in bagasse as substrate in 0.4 per cent in dextrose as carbon source recorded highest activity of $0.689Uml^{-1}$ than extracellular β-glucosidase activity in 0.4 per cent fructose ($0.403Uml^{-1}$). The minimum intracellular activity of $0.073Uml^{-1}$ was recorded in 0.3 per cent fructose and extracellular activity ($0.110Uml^{-1}$) in 0.4 per cent sucrose as carbon source. The present result is at par with the result of Camassola and Dilon, 2007 who evaluated the production of cellulase enzyme complex by *Penicillium echinatum* grown on pre-treated sugarcane bagasse and wheat bran in solid state fermentation. They obtained a highest amounts of filter paper activity of $58.95\pm1.90Ugdm^{-1}$, â-glucosidase activity of $58.95\pm2.58Ugdm^{-1}$ and endoglucanase activity of $282.36\pm1.23Ugdm^{-1}$ in the mixture of pre-treated sugarcane bagasse on the 4th day of incubation.

### Conclusion:

The incorporation of cheap carbon sources such as sugarcane bagasse into the media for the production of lignocellulolytic enzymes could help to decrease the production costs of enzymatic complexes that can hydrolyse lignocellulose residues for the formation of fermented syrups, thus contributing to the economic

production of bioethanol. Thus, it can be deduced from the present investigation, that the cellulolytic fungal strains like *Aspergillus* spp. can be exploited as an efficient candidate for production of cellulase enzyme complex which can be utilized for industrial applications.

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