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Production of Cellulase Enzyme by *Chaetomium* sp. using Wheat Straw in Solid State Fermentation

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Abstract: The aim of the present study was production of cellulase enzyme using wheat straw as substrate from *Chateomium* sp. under Solid State Fermentation (SSF) condition. The culture parameters, such as duration of incubation, incubation temperature, pH, carbon and nitrogen sources and additives, were optimized for enhancing cellulase yield. The optimal level of each parameter for maximum cellulase production by the fungus was determined. Results showed that cellulase production was highest with wheat straw containing production medium supplemented with sucrose, malt extract and $CaCl_2$ and the incubation temperature, time and pH were 35°C, 3rd day and 6.5, respectively. The importance of cellulase enzyme in industries cannot be over emphasized. The crude enzyme when purified may serve the importance of this enzyme in both refining and deinking of recycled papers.

Key words: Agricultural wastes, additives, carbon and nitrogen sources, cellulase enzyme, *Chaetomium* sp.

INTRODUCTION

Solid State Fermentations (SSFs) are those processes in which microbial growth and products formation occurs on the surfaces of water insoluble materials in the absence of free water. The most important benefit of SSF is low operating cost, space requirement and easier as well as cheaper product recovery. The process emphasizes the need to maintain a static substrate and absence of free water. Literature highlighting the production of several industrially important enzymes using SSFs from several agro industrial wastes and byproducts exploring microorganisms are available (Chahal *et al*., 1996; Kaur and Satyanarayana, 2004; Singhania *et al*., 2006; Chandra *et al*., 2007; Sherief *et al*., 2010). The most commonly used microorganisms in solid state bioprocessing are fungi (Lakshmikant and Mathur, 1990; Milala *et al*., 2005).

Cellulases (a complex multienzyme system) are carbohydrases that degrade cellulose by cleaving β-1, 4-glycoside linkage into simple soluble reducing sugars (Bhat, 2000). Cellulases

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enzymes are widely used in the bioconversion of waste cellulose to compounds of economic importance such as glucose, cellobiose and bioethanol (Smith, 1996; Ohmiya *et al*., 1997). Several studies were carried out to produce cellulases under suitable conditions from different cellulolytic organisms including fungi such as *Trichoderma, Penicillium*, *Aspergillus*, *Myrothecium*, *Fusarium*, *Chaetomium* species etc. (Hoffman and Wood, 1985; Chahal *et al*., 1996; Krishna *et al*., 2000; Hayat *et al*., 2001; Milala *et al*., 2005; Singhania *et al*., 2006; Chandra *et al*., 2007; Immanuel *et al*., 2007; Raza and Rehman, 2009; Sherief *et al*., 2010) and bacteria like *Pseudomonas*, *Cellulomonas*, *Bacillus*, *Micrococcus* and *Clostridium* species etc. (Nakamura and Kppamura, 1982; Immanuel *et al*., 2006).

Chaetomium is a fungal genus containing around 80 known species. The most widespread ones are *C. atrobrunneum*, *C. funicola*, *C. globosum*, *C. thermophilum* and *C. strumarium*. It is a filamentous fungus commonly found in soil, rock, air, decaying plant materials especially woody or straw like materials and from herbivore dung.

Considering the biotechnological importance of fungi in the enzyme industries, the present work was carried out to produce cellulase from *Chaetomium* sp. using a cost effective and easily available source (wheat straw) in solid state fermentation conditions. Furthermore, an attempt was made for determination of some factors that would result in optimal production of cellulase.

MATERIALS AND METHODS

Materials

Chemicals

All chemicals were of analytical grade and obtained from CDH and HiMedia. Agar-agar was purchased from Merck.

Substrate

Wheat straw was obtained commercially from the local vendors. After drying at 70°C wheat straw was ground in an electric grinder and stored in polythene bags for subsequent use as fermentation medium.

Fungal Culture

Fungal strain was isolated from decayed wheat leaves taken from the wheat field near MIET, Meerut and cultured on PDA media. A pure culture was raised by subculturing and identified as *Chaetomium* sp. Purified culture was maintained on PDA slant at 4°C. The present work was conducted during Sep. 2009 to Jan. 2010.

Solid State Fermentation

Solid State Fermentation (SSF) was carried out using a 250 mL erlenmeyer flask containing production medium (2% w/v wheat straw). After sterilization (121°C/20 min) inoculated with 10 mL aliquots of conidia suspension (approx. 10^7 spore/gm dry substrate) which was obtained from a 7 day old PDA slant.

Production Studies

The production of cellulase was followed for seven days; the contents of the flasks were harvested at regular intervals (24 h). The culture was centrifuged at 8000 rpm for 10 min at 4°C. The resultant clear supernatant used as a crude enzyme.

Optimization of Parameters for Enzyme Production

Effect of Incubation Period

Chaetomium sp. was inoculated into production medium and incubated at 30°C for seven days in stationary conditions. The cellulase activity was measured at regular intervals of 24 h and the period of maximum enzyme production determined.

Effect of Temperature

The effect of different incubation temperatures (30, 35, 40, 45, 50, 55 and 60°C) on cellulase production was screened.

Effect of pH

Chaetomium strain was inoculated into production medium with the pH ranging from 5 to 8. The inoculated media were incubated at 35°C for three days.

Effect of Various Carbon and Nitrogen Sources

Various carbon sources (2% w/v), including glucose, fructose, sucrose, cellulose, lactose, maltose and galactose on cellulase production were screened. Similarly, effect of various nitrogen sources were studied by supplementing the production media with various organic (1%) and inorganic (0.1%) nitrogen sources viz., malt extract, yeast extract, beef extract, peptone, tryptone, NH_4Cl, $(NH_4)_2SO_4$ and NH_4NO_3.

Effect of Various Additives

Each 250 mL flask containing production medium was supplemented with different additives (0.1%w/v) viz., NaCl, KCl, $CaCl_2$, $MnCl_2$, $FeCl_3$ and $MgCl_2$.

Enzyme Assay

The cellulase enzyme was assayed by measuring the amount of glucose released from the substrate following the secretion of cellulase by the organism. The cellulase activity was determined by DNSA method (Miller, 1959).

RESULTS

Production of Cellulase by *Chaetomium* sp.

The production profile of cellulase on wheat straw containing medium was studied. The proper combination of various cultural conditions was established in order to achieve maximum cellulase production.

Effect of Incubation Period

Fermentation was carried out for seven days to determine the optimum fermentation period. The data were expressed in Fig. 1. It was found that maximal production of cellulase (42.60 IU mL^{-1}) was obtained on 3rd day of incubation. Furthermore, incubation resulted in the decline in enzyme activities up to seven days.

Optimum Temperature and pH

The activity of the enzyme was measured at different temperature (30 to 60°C) and shown in Fig. 2. Maximum production was found at temperature of 35°C. Similarly effect of pH on cellulase production was tested by growing *Chaetomium* sp. in pH range of 5 to 8. The data shown in Fig. 3 revealed that maximum activity (30.1 IU mL^{-1}) was at pH 6.5.

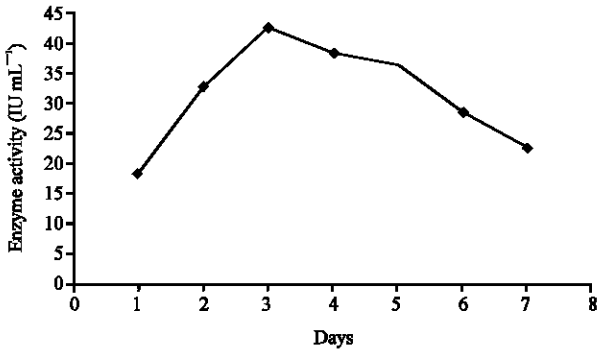

Fig. 1: Effect of incubation time on enzyme production

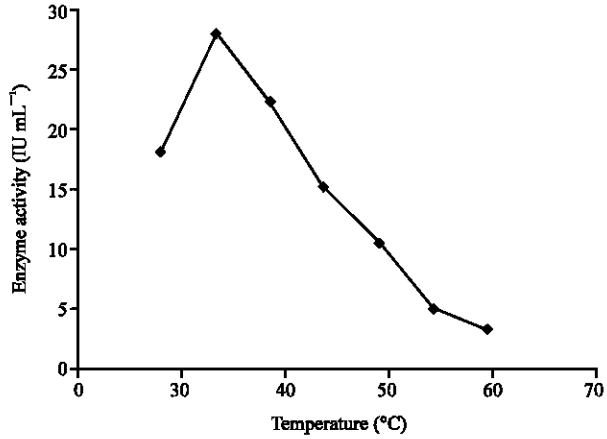

Fig. 2: Effect of temperature on enzyme production

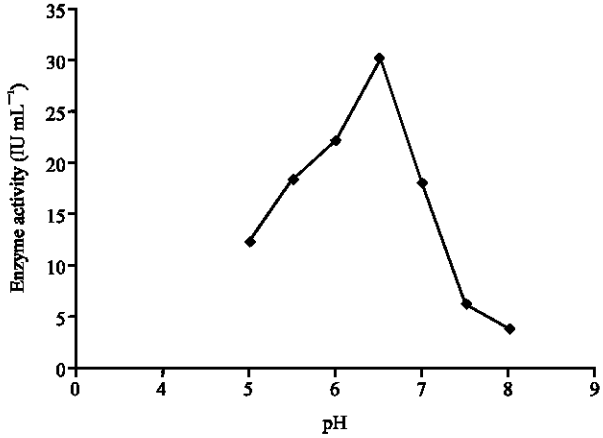

Fig. 3: Effect of pH on enzyme production

Effect of Carbon and Nitrogen Sources

Addition of different C-sources (glucose, fructose, sucrose, cellulose, lactose, maltose and galactose) to wheat straw containing production medium, on the cellulase production

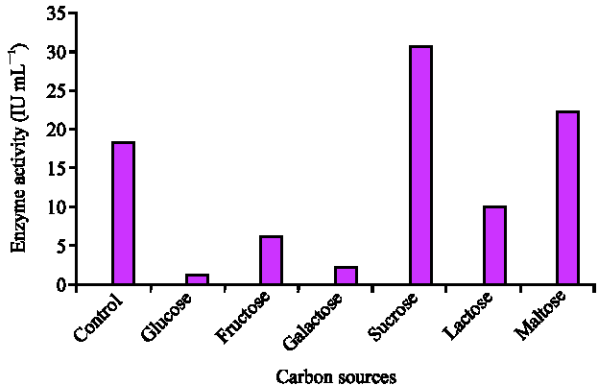

Fig. 4: Effect of carbon sources on enzyme production

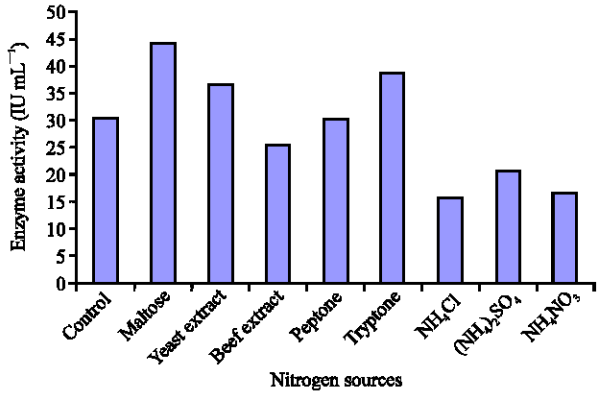

Fig. 5: Effect of nitrogen sources on enzyme production

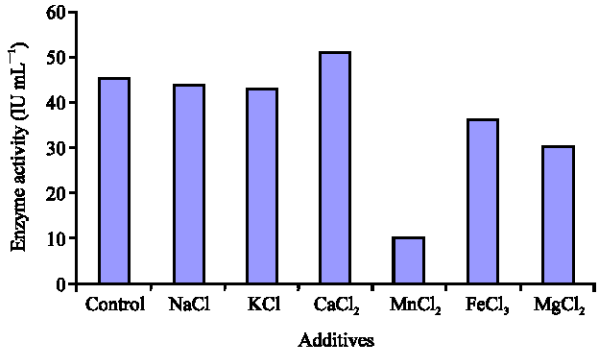

Fig. 6: Effect of additives on enzyme production

was studied. The supplementation of sucrose to the production medium resulted in marked increase in cellulase activity producing 30.8 IU mL^{-1} as compared to control as shown in Fig. 4. The enzyme production, however, was strongly repressed in the presence of glucose (1.26 IU mL^{-1}).

As shown in Fig. 5 among the various N-sources (malt extract, yeast extract, beef extract, peptone, tryptone, NH_4Cl, $(NH_4)_2SO_4$ and NH_4NO_3) used in the production medium the

presence of malt extract was found to support maximal production of cellulase (44.3 IU mL^{-1}) followed by Tryptone (38.6 IU mL^{-1}) and then by yeast extract (36.3 IU mL^{-1}). Minimum enzyme activity was reported as 15.6 IU mL^{-1} with NH_4Cl.

Effect of Various Additives

Results shown in Fig. 6 indicated that among all additives (NaCl, KCl, $CaCl_2$ $MnCl_2$, $FeCl_3$ and $MgCl_2$) $CaCl_2$ resulted in max production of enzyme (50.8 IU mL^{-1}). Addition of other additives resulted in decrease enzyme level. The least production of cellulase was observed in production medium supplemented with $MnCl_2$ (10.2 IU mL^{-1}).

DISCUSSION

Microflora is the easy and very important key source for the production of several biologically active compounds such as enzymes and biomass/single cell proteins, even though, several microorganism are easily grown on several biological waste products utilizing them as protein and energy sources for synthesis of several useful products.

Cellulase from microorganisms is one of the key enzymes on account of their industrial applications (Ohmiya *et al*., 1997; Bhat, 2000). Cellulase production of different organisms has received more attention and is exploited for generation of cellulolytic enzymes with the use of cheaply available lignocellulosic residues (Lakshmikant and Mathur, 1990; Kaur and Satyanarayana, 2004; Chandra *et al*., 2007). The cellulase production by *Chaetomium* sp. was ably supported by wheat straw, which is a cheap and readily available carbon source, similar findings were reported by several other workers (Raza and Rehman, 2009; Sherief *et al*., 2010). The high cost of production of these enzymes has hindered the industrial application of cellulase. One of the approaches to overcome this hindrance is to make continuous search for organisms with secretion of cellulase enzyme in copious amounts and to optimize enzyme production with them. *Chaetomium* sp. a dematiaceous filamentous fungi has not been exploited for the production of industrial enzymes, hence, the present studies was carried out with a view to produce and characterize the extracellular cellulase from *Chaetomium* sp. using wheat straw.

Chaetomium sp. used in this study produced cellulase under solid state fermentation conditions. the importance of SSF for enzyme production is also evident by earlier work done with different microorganism (Chahal *et al*., 1996; Singhania *et al*., 2006; Chandra *et al*., 2007; Sherief *et al*., 2010). There was gradual increase in the production of cellulase with maximum level observed at 3rd day of incubation at 35°C. Similarly reports exists for the production profile of cellulase from *Chaetomium thermophiles* (Hayat *et al*., 2001; Chandra *et al*., 2007; Raza and Rehman, 2009), *Aspergillus* (Nakamura and Kppamura, 1982; Milala *et al*., 2005; Chandra *et al*., 2007) and *Trichoderma* (Krishna *et al*., 2000; Singhania *et al*., 2006). The instability of cellulase enzyme at very low or very high pH values as shown in Fig. 3, is due to the fact that they are proteins which are generally denatured at extreme pH values (Steiner *et al*., 1994).

Among the various carbon sources sucrose was proved to be the best carbon source produced 30.8 IU mL^{-1} cellulase. As compared to inorganic sources, organic nitrogenous sources showed higher enzyme production which is in harmony with previous report of Umikalsom *et al*. (1997). Among nitrogen sources malt extract yielded maximum enzyme production (44.3 IU mL^{-1}). This indicates that inorganic nitrogenous sources did not induce enzyme production. It was reported that maximum cellulase yield was obtained in presence of peptone as nitrogen source followed by yeast extract. Urea, KNO_3 and $(NH_4)_2SO_4$ in wild strain of *Chaetomium globosum* (Umikalsom *et al*., 1997).

Various additives (except $CaCl_2$) supplemented in the production medium had not found to enhance the cellulase production. Similar results were also reported in *Streptomyces* strain (Ishaque and Kluepfel, 1980), *Trichoderma reesei* (Krishna *et al*., 2000) and *Nectria catalineasis* (Pardo, 1996).

CONCLUSION

From the above experiments it was concluded that the *Chaetomium* sp. expressed maximum cellulolytic activity in SSF condition on 3rd day incubation in a medium containing wheat straw supplemented with sucrose, malt extract and $CaCl_2$ at 35°C (pH 6.5). The results of the investigation highlight the industrial potential of the cellulases from *Chaetomium* spp. because of its yield, stability and economic production using agro residue such as wheat straw.

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