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Production of Endoglucanase by *Fusarium solani* Grown in Solid-State Fermentation

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Corn cob is an abundant lignocellulosic agricultural waste. In this investigation, the production of endoglucanase from this lignocellulosic biomass under solid state fermentation was investigated. The effects of various process parameters, such as initial moisture content, initial culture pH, amount of substrate, incubation period on endoglucanase production by a mesophilic fungus *Fusarium solani*, were studied. With an initial moisture content of 70 %, a pH of 5.0, maximum endoglucanase activity (312 ± 16 U/gds) was obtained with 15 g of corn cobs after 96 h. Addition of nitrogen sources further enhanced the endoglucanase production.

Key Words: Cellulases, *Fusarium solani*, Solid state cultivation, Corn cobs.

INTRODUCTION

Cellulose, a homopolymer of β-1,4-linked anhydrous glucose units, is the most abundant organic compound in the biosphere and it is a kind of renewable energy on earth. The cellulosic biomass is obtained from various agricultural wastes and conversion of this biomass into useful products such as sugar, ethanol and methane is an attractive option for energy production and remedy to environmental pollution[1].

Microbial conversion of cellulosic biomass involves the synergistic action of 3 major enzymes *i.e.*, endo-β-1,4-glucanase (E.C. 3.2.1.4), exo-β-1,4-cellobiosidase (E.C. 3.2.1.91) and β-glucosidase (3.2.1.21). Endoglucanase acts in a random fashion, cleaving β-linked bonds within the cellulose molecule; cellobiohydrolase removes cellobiose units from the non-reducing ends of the cellulose chain and β-glucosidase splits cellobiose and cellooligosaccharides into glucose[2]. The major industrial applications of cellulases are in the textile industry for polishing of fabrics and producing stonewashed look of denims, as well as in house-hold laundry detergents for improving softness and brightness of fabrics[3]. Besides, they are used in various industries, including the food, brewery and wine, animal feed, agriculture, pulp and paper industry as well as in the bioconversion of renewable cellulosic biomass to commodity chemicals[4-7].

Enzyme production is a growing field of biotechnology and various agricultural substrates/byproducts has been used successfully by different microorganisms to produce cellulases under solid state cultivation[8-11]. Solid state fermentation is an attractive process for industrial enzymes production and has many advantages over submerged fermentation. Solid state fermentation compared to submerged fermentation is more simple, requires low capital, has superior productivity, reduced energy requirement, uses less water and produces lower waste water, has easier control of bacterial contamination and requires low cost of downstream processing[12-14].

Cellulases have been produced by many filamentous fungi[15] and especially the fungus *Trichoderma reesei* has been studied in great detail due to its ability to secrete large amounts of cellulases[16]. However, it is of interest to explore new microorganisms for enhanced production of cellulases. In this context we use *Fusarium solani* strain to study the production of endoglucanase using corncobs as substrate under solid state fermentation. The effect of different process parameters such as initial moisture level, initial pH, amount of substrata, incubation period and some additives were studied.

EXPERIMENTAL

Substrate: Corn cob was used as substrate for the production of endoglucanase. It was dried in an oven at 105 °C to a constant weight and then chopped in a laboratory hammer mill to a particle size *ca.* 40 mm.

Organism and culture conditions: The fungus used in this study, *Fusarium solani* was obtained from National Fungal Culture Collection of Pakistan (NFCCP), Department of Plant Pathology, University of Agriculture, Faisalabad. The stock culture was maintained on potato dextrose agar (PDA) slants at 4 °C and sub-cultured fortnightly.

Inoculum preparation and growth conditions: Inoculum was prepared by transferring spores from 5-6 d old slant culture, into 500 mL Erlenmeyer flask containing 150 mL of sterile Vogel's medium as described elsewhere[17,18]. Solid state fermentation was carried out in 100 mL Erlenmeyer flasks containing 5.0 g of corn cob with different volumes of mineral medium. The composition of the mineral medium was (g/L): trisodium citrate, 2.5; KH_2PO_4, 5.0; NH_4NO_3, 2.0; $(NH_4)_2SO_4$, 4.0; $MgSOg.7H_2O$, 0.2. Flasks were plugged with cotton and sterilized by autoclaving for 15 min at 121 °C. After sterilization, the flasks were cooled and inoculated with 0.5 mL of mycelial suspension. The flasks were incubated at 30 ± 1 °C and initial pH 5 under still culture conditions for desired time period. The contents of the flasks were gently shaken (for mixing) after every 12 h.

The solid state fermentation of corn cob was optimized by varying process parameters like initial moisture content, initial growth pH, incubation period, substrate amount and the effect of nitrogen additives. The traditional classical method involved varying one parameter at a time by maintaining pre-optimized solid state fermentation conditions.

Enzyme extraction: The fermented biomass was soaked in distilled water (1:20, w/v). The mixture was allowed to stand for 1 h and the extracts were obtained by filtering the mixtures through muslin cloth. The filtrates were centrifuged at 18,000 rpm (39,200 x g) for 0.5 h at 4 °C to remove the suspended particles. The clear supernatant was used as enzyme source.

Enzyme assay: Endoglucanase activity was determined using 1 % (w/v) carboxymethyl cellulose (CMC) as substrate prepared in acetate buffer (50 mM, pH 5.0). The reaction was initiated by incubating 1 mL of the enzyme with 2 mL of the substrate for 20 min at 60 °C. The reaction was terminated, thereafter, by addition of 3,5-dinitrosalicylic acid reagent (DNS) followed by boiling the contents[19] for 10 min and the developed colour was read at 540 nm using U-2001 spectrophotometer (Hitachi). One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of glucose equivalent per min under the above assay conditions. All enzyme activities were expressed as units of enzyme per gram of dry weight of substrate (U/gds).

All the experiments were performed in triplicate and the results are expressed as mean ± SD.

RESULTS AND DISCUSSION

Corn cob is among the most common agro-industrial by-products produced in large quantities. An attempt has been made to use this by-product as a potential substrate for solid state fermentation using a mesophilic fungus *Fusarium solani*.

In order to study the effect of moisture content, the substrate, corn cob was moistened with an initial moisture content of 40, 50, 60, 70 and 80 % using mineral solution; values being set before autoclaving. Maximum endoglucanase yield (215 ± 13 U/gds) was achieved at the initial moisture content of 70 % after 96 h at 30 ± 1 °C. The lowest enzyme activity (125 ± 8 U/gds) was with 40 % as the initial moisture content (Fig. 1). The critical importance of moisture content in solid-state fermentation and its influence on the biosynthesis and secretion of enzyme can be attributed to the interference of moisture in the physical properties of the solid particles. High substrate moisture results in decreased porosity which in turn prevents oxygen penetration[20]. At the same time, low moisture level lead to poor microbial growth and poor accessibility to nutrients. It has been observed that high moisture content enhanced fungal growth and enzymes production when lignocellulosic substrates were used as carbon source in solid state fermentation[9,21].

The effect of initial culture pH of the medium on the production of endoglucanase was determined by growing *F. solani* on corn cobs using different levels of pH (3-8) of the moistening medium. The results shown are shown in Fig. 4. It was observed that maximum activity of endoglucanase (255 ± 13 U/gds) was obtained at pH 5.0 after 96 h of incubation period at 30 ± 1 °C. Optimal pH is very important for growth of microorganism and its metabolic activities. As the metabolic activities of the microorganism are very sensitive to changes in pH, endoglucanase production by *F. solani* was found to be affected by initial culture pH. The obtained results are similar to those reported for other fungal cellulases[9,22,23].

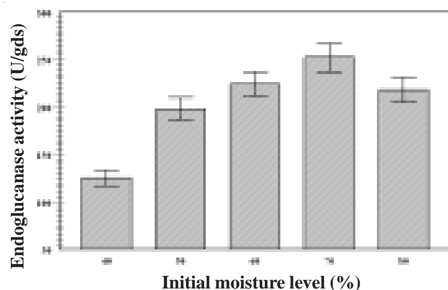

Fig. 1. Effect of initial moisture content on endoglucanase production

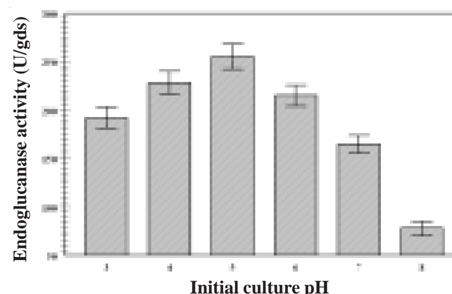

Fig. 2. Effect of initial culture pH on endoglucanase production

Solid state fermentation of corn cobs was carried out for different incubation periods (24-144 h) at pre optimized culture conditions. *F. solani* produced high titres of enzyme (272 ± 15 U/gds) after 96 h incubation (Fig. 3). The production of endoglucanase decreased after 96 h. This fact is probably due to lack of nutrients of the medium which affect organism growth and ultimately enzyme biosynthesis. It is another important parameter that has to be controlled which varies from organism to organism[12].

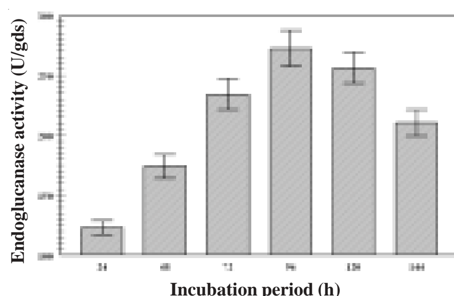

Fig. 3. Effect incubation period on endoglucanase production

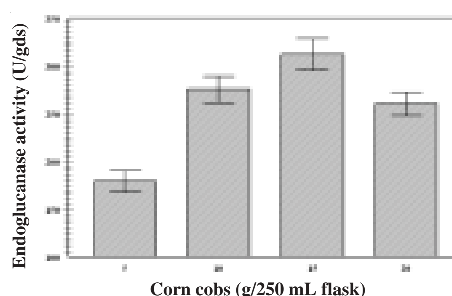

Fig. 4. Effect substrate amount on endoglucanase production

The level of substrate is important in solid state fermentation especially in tray processes. The level of substrate also influences the porosity and aeration of the substrate in flasks. Different amounts (5, 10, 15 and 20 g) of corn cobs were tried to study their effect on endoglucanase production in 250 mL conical flasks. Maximum enzyme activity (312 ± 16 U/gds) was observed in flask containing 15 g of corn cobs (Fig. 4). Different organic carbon sources were added separately to the solid substrate medium as additives at the concentration of 1 % (w/w) to assess their effects on endoglucanase production. Addition of different carbon sources inhibited production of the enzyme. When different nitrogen sources (urea, peptone, cotton seed meal and yeast extract) were tested, the results showed that endoglucanase production was higher than the control (Fig. 5). Maximum activity (336 ± 12 U/gds)

was observed with peptone. These observations are in accord with those of Grajeck[24] and Gomes *et al.*[25] who also observed a similar effect of organic nitrogen additives on cellulases production.

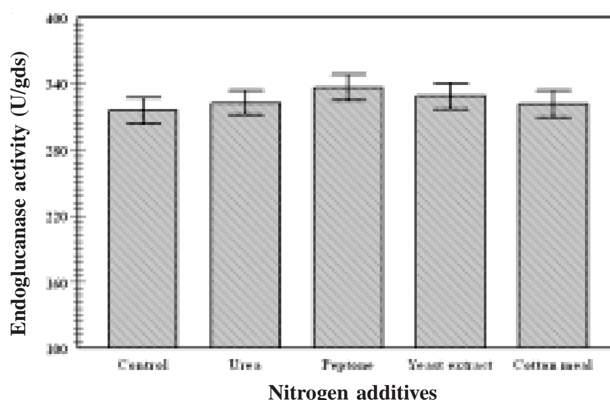

Fig. 5. Effect nitrogen additives on endoglucanase production

Conclusion

Based on the results, it can be concluded that *F. solani* can be cultivated under solid state fermentation for the production of cellulolytic enzyme using corn cobs, an agricultural waste, enriched with minerals and nitrogen additives. The technique of solid state fermentation would help in producing enzyme in a short time and consequently may help in reducing the cost of endoglucanase production.

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