

Optimization of process parameter for protease production by *Fusarium oxysporum*

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ABSTRACT

The investigation was under taken to study process parameter for protease production by *Fusarium oxysporum* isolated from diseased seed of groundnut. The protease production was studied using gram bran as a substrate and in relation to different incubation time, temperature, moisture level and pH. Maximum protease production was observed on 35°C temperature, 5.5 pH and 120 h of incubation and initial moisture level 50%.

Key words: Process parameter, *Fusarium oxysporum*, solid-state fermentation, gram bran.

INTRODUCTION

Protease is one of the most important commercial enzymes that catalyze hydrolytic reaction in which protein molecules are degraded to peptides. It used in food processing, detergents, dairy industry and leather making (Negi and Benerjee., 2006). The protease enzyme constitutes two thirds of total enzymes used in various industries which need to increase production. (Gupta *et al.*, 2002). Protease occurs widely in plant and animals, but commercial proteases are produced exclusively from microorganism. Molds of the genera *Aspergillus*, *Penicillium* and *Rhizopus* are especially use for producing protease. (Sandhya *et al.*, 2005). Protease is extracellular enzymes that can be produced by both submerged fermentation and solid-state fermentation. Solid-state fermentation is especially suited to growth of fungi because of their lower moisture requirement compared with bacteria (Ogawa *et al.*, 1995). Solid state fermentation is simple, low cost, and provides high yields of appropriate enzymes (Wang *et al.*, 2005).

For production of enzyme and its industrial use, isolation and characterization of new promising strains using cheap carbon and nitrogen source is a continuous process. The present study was undertaken to increase protease production of

Fusarium oxysporum by using gram bran as a substrate and optimizing the process parameter such as moisture level, temperature, pH, and incubation time for maximum protease production.

MATERIAL METHODS

Fusarium oxysporum was isolated from diseased seed of groundnut by blotter technique (DeTempe., 1963) and identified (Alexopoulos., 1962). The pure culture was maintained on Czapak-Dox agar containing (g/l). Sodium nitrate 2, KCl 0.5, K_2HPO_4 1, $FeSO_4 \cdot 7H_2O$ 0.01, Sucrose 3.0, pH, 5.5. for further use. The Substrate was prepared by milling gram bran which was then sieved through 20-40 mesh screens to obtain a particles having diameter between 0.42 and 0.85mm. Fifty gram of substrate was supplemented with 0.83 g K_2HPO_4 and 0.16g $MgSO_4$ and 10 ml of distilled water, autoclaved for 15 min. at 121°C.

Fermentation was initiated by preparing inoculum of spore suspension of 7 day old culture of *Fusarium oxysporum* grown on Czapak-Dox agar. The spores were collected in sterile water (Villegas *et.al.*, 1993). A 250 ml flask containing 20gm sterilized 50% moistened substrate was inoculated with spore suspension under aseptic condition and incubated at 30°C for 7 days. After every 12 h of

interval 1 gm substrate was harvested from flask and transferred to a test tube containing 5ml phosphate buffer. The contents were homogenized and centrifuge at 2000 rpm for 30 min to remove all particulate matter. The cultural filtrate was filtered through Whatman filter paper and used for enzyme assay.

The protease activity was assayed as suggested by Keay and Wridli (1970). To 1ml of culture filtrate 1 ml of 2% casein solution was added and the mixture was incubated at 37°C for 10 min. the reaction was terminated by adding of 2 ml 0.4 M TCA (Trichloro acetic acid), again incubated for 20 min. and filtered through Whatman filter paper. One ml of the filtrate was added to 5 ml of sodium carbonate (0.4M) and 1 ml of Folin-Ciocalteus's reagent and incubated at 37°C for 30 minutes. The absorbance was measured at 660 nm using a tyrosine standard. A unit of protease activity was defined as the amount of enzyme liberated 1ug of tyrosine from substrate (Casein) per minute under assay conditions and reported in terms of protease activity per gram dry fermented substrate.

Effect of incubation period

The effect of incubation period on protease was determined by incubating production medium for different incubation periods in day i.e. 24,48,72,96, 120 and 144h.

Effect of initial moisture content

Initial moisture of substrate plays important role in mold growth and enzyme production in solid-state fermentation. To determine the effect of moisture substrate were moistened at 45, 50, 55, and 60% with distilled water. The fermentation was carried out for 120h at 35°C.

Effect of incubation temperature

The inoculated substrate was incubated at different temperature i.e. 25, 35, 40, 45, and 50°C to observe the effect of temperature, by keeping all other condition at their optimum level.

Effect of initial pH

In general practice, the pH in solid-state fermentation is almost never controlled during fermentation, only the initial pH of the substrate is adjusted before inoculation. To evaluate production

of protease initial pH of substrate was adjusted at 5, 5.5, 6, 6.5 and 7pH with 1 N HCL or 1N NaOH. The fermentation was carried out at 35°C to study their effect on enzyme production.

RESULTS AND DISCUSSION

Proteases are extracellular enzyme that can be produced by both submerged fermentation and solid state fermentation. But solid state fermentation is especially suited to growth of fungi because of their lower moisture requirements. The production of enzyme can be enhanced by optimizing process parameter of solid state fermentation.

The result of present study shows that when *Fusarium oxysporum* was grown on gram bran as a substrate under solid-state fermentation condition. It secreted protease enzyme, but the amount of protease production varied with incubation time and maximum enzyme production was observed at 120 days of incubation. (Fig.1). A gradual decrease in enzyme units was observed with increasing incubation period clearly indicate that enzyme role as a primary metabolite, being produced in the log phase of the growth of the fungus for utilization of nutrients (proteins) present in solid substrate. (Alagarsamy *et al.*, 2006). Increased in moisture level is believed to reduced the porosity of the gram bran, thus limiting oxygen transfer. While lower moisture content causes reduction in the solubility of nutrients of the substrate and lower degree of swelling. Hence, an optimal level of moisture is required for maximum enzyme productivity. A maximum enzyme titer was found at the initial moisture level 50% (Fig.2).

The optimum temperature for maximum proteases production was found at 35°C (Fig. 3). A decrease in the yield of protease was observed when the incubation temperature was higher or lower. Effect of initial pH of the substrate on protease production showed that at pH 5.0, there was less enzyme production. As the pH was increased protease production increased gradually and reached to maximum at pH 6.5, a further increased in pH resulted in decreased of protease production (Fig.4).

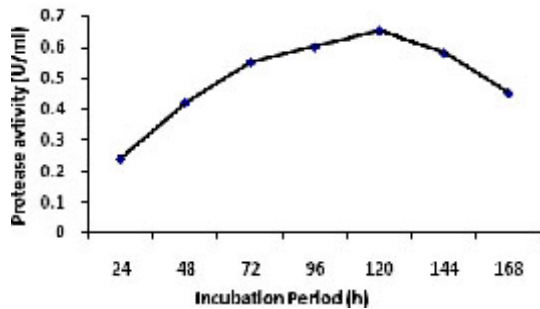


Fig. 1: Effect of incubation time on enzyme production

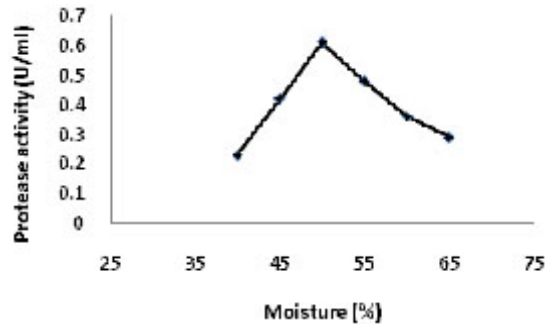


Fig. 2: Effect of initial moisture on enzyme production

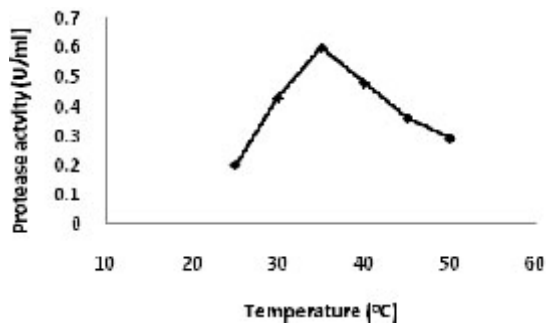


Fig. 3: Effect of different temperature on enzyme production

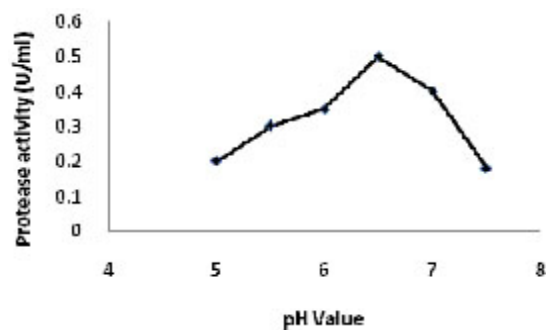


Fig. 4: Effect of different pH on enzyme production

CONCLUSION

A strain of *Fusarium oxysporum* produced maximum protease when grown on gram bran as a substrate under solid-state fermentation. The enzyme production was enhanced by optimizing process parameter. Maximum protease production

was observed at 120h of incubation with initial moisture 50% and pH 6.5 at 35°C temperature. Thus gram bran as a substrate and all the above mentioned optimized condition under solid state-fermentation increased the protease production and can be exploited for large scale production of protease enzyme by using agroindustrial waste.

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