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Enhanced Amylase Production by *Fusarium solani* in Solid State Fermentation

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Abstract. The present study illustrates the investigation carried out on the production of amylase by *Fusarium* species under solid state fermentation. All the tested *Fusarium* species were capable of producing amylase. A selected *F. solani* isolate SY7, showed the highest amylase production in solid state fermentation. Different substrates were screened for enzyme production. Among the several agronomic wastes, wheat bran supported the highest yield of amylase (141.18 U/g of dry substrate) after 3 days of incubation. Optimisation of the physical parameters revealed the optimum pH, temperature and moisture level for amylase production by the isolate as 8.0, 25 °C and 70%, respectively. The above results indicate that the production of amylase by *F. solani* isolate SY7 could be improved by a further optimisation of the medium and culture conditions.

Keywords: agro-industrial wastes, α-amylase, *Fusarium* spp., solid state fermentation

Introduction

α-amylase (EC 3.2.1.1, a-1,4-glucan-4-glucano-hydrolase), is an extra cellular enzyme, which catalyses the endocleavage of the a-1,4-glycoside linkages and the release of short oligosaccharides and a-limit dextrin. This enzyme is used commercially for the production of sugar syrups from starch, which consist of glucose, maltose, and higher oligosaccharides (Reddy *et al*., 2003). It is also extensively used in starch of liquefaction and paper, food, pharmaceutical and sugar industries. Although, amylases can be obtained from several sources, such as plants and animals, the enzymes from microbial sources generally meet industrial demand (Nwagu and Okolo, 2011; Pandey *et al*., 2001).

Fungal amylases are preferred to plant enzymes due to their short growth period, higher productivity and thermostability (Mishra *et al*., 2008). However, fungal growth and amylase production are dependent on growth conditions, such as type and concentration of carbon and nitrogen sources, metal ion requirement, pH and temperature of growth (Ghasemi *et al*., 2010; Cherry *et al*., 2004). Though many microorganisms can grow on a wide range of carbon and nitrogen sources, it is economically more viable to utilise the cheap and easily available resources as substrates for amylase production (de Castro and Sato, 2013; Pandey *et al*., 2001). Industrial enzymatic hydrolysis is influenced by a number of factors amongst which are environmental conditions of pH, temperature and presence of metal ion (Riaz *et al*., 2007).

Fungal amylase is preferred for use in formulation for human or animal consumption involving application under acidic condition and around 37 °C. Studies on fungal amylase especially in the developing countries have concentrated mainly on filamentous fungi probably because of the ubiquitous nature and non-fastidious nutritional requirements of these organisms (Padmini *et al*., 2012; Guimaraes *et al*., 2006).

Fusarium is a large genus of filamentous fungi, and most of *Fusarium* species are harmless saprobes and relatively abundant members of the soil microbial community (Alazem, 2007; Summerell *et al*., 2001; Onyika *et al*., 1993). These species have the ability to produce different enzymes under fermentation conditions (Kikot *et al*., 2010; Pekkarinen *et al*., 2000). Cereal grains showed cavities and furrows in endosperm of starch granules, evidencing damage caused by amylases (Jackowiak *et al*., 2002). This ecological habitat of the fungus, however, implies that *Fusarium* would be a useful resource of extracellular enzymes. Solid state fermentation (SSF) is widely established for the production of enzymes from filamentous fungi (Zaferanloo *et al*., 2014; de Castro and Sato, 2013). Morphology and physiology of these molds enable them to penetrate and colonise various solid substrates (Vijayaraghavan *et al*., 2011). SSF utilises various agroindustrial wastes as substrate that acts both physical support and source

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of nutrients (Pandey *et al*., 2001). In addition, the use of SSF for enzyme production has many advantages over submerged fermentation due to its simple technique, low capital investment, lower levels of catabolite repression and better product recovery (Considine *et al*., 1989). However, filamentous fungi are the best adapted for SSF. The hyphal mode of fungal growth and their good tolerance to low water activity and high osmotic pressure conditions make fungi efficient and competitive in natural microflora for bioconversion of solid substrates (Padmini *et al*., 2012; Pandey *et al*., 2001).

The objectives of the present study were, (i) to investigate the ability of *Fusarium* species to produce amylase, and (ii) to find out optimum condition for enzyme production under solid state fermentation.

Materials and Methods

Microorganism. *Fusarium* spp., isolates were obtained from wheat seeds showing disease symptoms from different locations in Syria. They were identified morphologically according to Nelson *et al*. (1983). Emphasis was placed on selecting isolates that induce differential reactions on specific wheat genotypes (Alazem, 2007), leading to the selection of 21 monosporic isolates (eight belonging to *F. culmorum*, six to *F. verticillioides*, four to *F. solani* and three to *F. equiseti*) used in this study (Table 1). The cultures were maintained on silica gel at 4 °C until needed.

Optimisation of cultural conditions. Enzyme production was carried out as reported by Bakri *et al*. (2008). The fermentation medium consisted of: (g/L) $Na_2HPO_4.2H_2O$ 10; KCl 0.5; $MgSO_4.7H_2O$ 0.15, and yeast extract 5, as a nitrogen source. The influences of different lignocellulosic materials *viz*., wheat bran, wheat straw, corn cobs hulls, soya cake and cotton seed cake on amylase production were tested (Fig. 1). Fresh fungal spores were used as inoculum and 1 mL spore suspension (containing around 10^6 spores/mL) was added to sterilised medium and incubated at 30 °C for 5 days. Various physical parameters such as pH (4, 5, 6, 7, 8 and 9), temperature (20, 25, 30, 35, 40 and 45 °C) and moisture level (50, 55, 60, 65, 70, 75, 80, 85 and 90%) were optimised by conventional methods for maximal enzyme production.

Extraction of amylase. Flasks were removed after cultivation and the enzyme was extracted by adding 25 mL of 0.1 M phosphate buffer (pH 5) to the cultures.

Table 1. Amylase production by *Fusarium* spp., in solid state fermentation

Isolate	Amylase (U/g)
F. culmorum	
SY1	45.5f
2	55.36d
3	54.6d
4	51.13e
6	76.13b
12	52.00e
13	40.40g
14	66.80c
F. verticillioides	
SY5	47.40f
9	51.40e
10	44.80f
15	54.30d
16	70.95bc
17	39.25g
F. solani	
SY7	118.35a
8	38.70g
11	43.50f
20	41.70f
F. equiseti	
SY22	33.60h
23	45.10f
24	58.80d

Values within a column followed by different letters are significantly different at P<0.001 according to Newman-Keuls test.

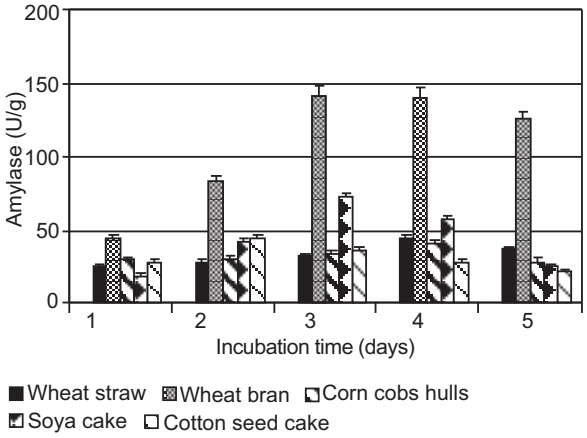

Fig. 1. Effect of lignocellulosic materials (wheat straw, wheat bran, soya cake and cotton seed cake) and incubation time on amylase production by *Fusarium solani* SY7.

The mixtures were shaken for 1.5 h on a magnetic stirrer. The supernatant was obtained by centrifugation (8000 × g for 15 min) followed by filtration through Whatman No. 1 filter paper and the filtrate was used as a crude enzyme preparation.

Assay of amylase: α-amylase activity was determined as described by Okolo *et al*. (2001). Reaction mixture contained: 1% soluble starch, 1.25 mL; 0.1 M acetate buffer (pH 5.0), 0.25 mL; and appropriately diluted crude enzyme extract, 0.25 mL. After 10 min of incubation at 50 °C, liberated reducing sugars (glucose equivalent) were estimated by the dinitrosalicylic acid method of Miller (1959). One unit (IU) of α-amylase is defined as the amount of enzyme that releases 1 μmol of glucose equivalent per min under the assay conditions and enzyme activity is expressed in terms of IU per gram dry fermented substrate.

Statistical analysis. The experiments were repeated twice and the means were analysed statistically with the analysis of variance (STAT-ICTF, 1988) with used to test for differences in amylase production among *Fusarium* isolates.

Results and Discussion

Amylase production from *Fusarium* species. The results showed that all the *Fusarium* species were capable of producing amylase. Significant differences ($P<0.001$) in the mean yield values were detected among isolates, with values being consistently higher in the isolates *F. solani* SY7 and *F. culmorum* SY6 (mean value 118.35 and 76.13 IU/g, respectively), whereas, low enzyme activity of 33.6 and 39.25 IU/g were detected for *F.equiseti* SY 22 and *F. verticillioides* SY17, respectively (Table 1). From this collection, *F. solani* SY7 isolate was selected for further studies.

Influence of some wastes on amylase production by *F. solani* SY7. Figure 1 shows that the highest amylase production (141.18 IU/g) was obtained on wheat bran after 3 days of incubation, whereas, soya cake and cotton seed cake exhibited low amylase production. These results might be attributed to the fact that the presence of readily available substrates has been noted to influence the biosynthesis of many extracellular enzymes *via* catabolite repression mechanism (Vijayaraghavan *et al*., 2011; Teodoro and Martins, 2000). Wheat bran was found to be the best substrate for α-amylase production by a thermophilic fungus *Humicola lanuginose* (Singh *et al*., 2009).

Influence of initial pH on amylase production by *F. solani* SY7. Since microorganisms are sensitive to the concentration of hydrogen ions present in the medium, pH is considered an important factor that determines the growth, morphology and product formation (Weiland, 1988). Figure 2 demonstrates that α-amylase production was significant over a wide range of pH values and was maximum at pH 8.0, which indicates that the selected isolate prefers alkaline conditions for better enzyme production. This was a rare occurrence because most fungal amylase required slightly acidic pH (4.5-6.0) (Okolo *et al*., 2001). Alva *et al.* (2007) observed two peaks optima in amylase production at initial pH 5.8 and 9.0 by *Aspergillus* sp., JGI 12. On the other hand, amylase production by *A. flavus* isolate FSS 60 was found to be best at pH 9.0 (Bakri *et al*., 2009). Thus, development of an optimal pH control strategy is helpful in obtaining higher enzyme productivity by the fungal strains.

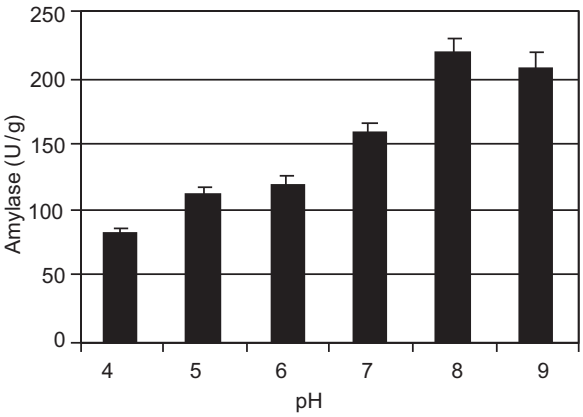

Fig. 2. Effect of pH degree on amylase produced by *Fusarium solani* SY7 grown on wheat bran under solid culture.

Influence of initial temperature on amylase production. Among the fungi, most amylase production studies have been conducted with mesophilic fungi within a range of temperature (25-37 °C) (Pandey *et al*., 2001). Figure 3 demonstrates the effect of temperature on α-amylase production, where the optimum temperature for maximum α-amylase production was 25 °C. Decrease in enzyme yield at lower or elevated temperatures resulted from the reduced metabolic activity and impaired action of the cell membrane of the fungus. The influence of temperature on the production of crude amylase showed that enzyme production decreased progressively with

increase in temperature (Fig. 3). Above 25 °C, there was a reduction in the amylase production. It is reported that the best amylase production in *A.niger* is at room temperature (Varalakshmi *et al*., 2009) and reported 30 °C be the best for amylase production by *Penicillium fellutanum* and *A. flavus*, respectively (Hernández *et al*., 2006; Okolo *et al*., 2001).

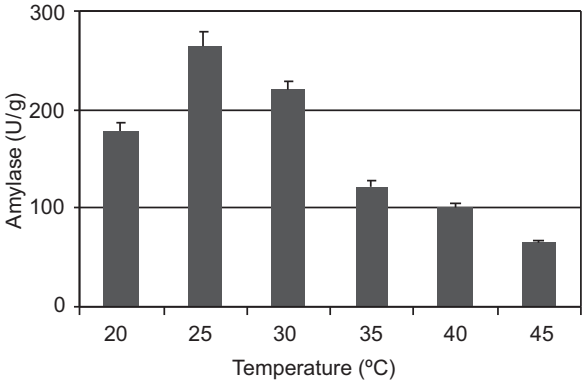

Fig. 3. Effect of temperature on amylase production by *Fusarium solani* SY7.

Influence of moisture level on amylase production. The results showed that the moisture level at 70% yielded the highest amylase production (Fig. 4). The critical importance of moisture level in SSF media and its influence on the biosynthesis of enzymes has been attributed to the interference of moisture in the physical properties of solid particles. Sodhi *et al*. (2005) reported that higher moisture level decreases porosity, changes wheat bran particle structure, promotes development of stickiness, reduces gas volume and exchange and decreases diffusion, which results in lowered oxygen transfer and reduction in enzyme production. On the other hand, lower moisture content reduces the solubility of nutrients present in solid substrate, decreases the degree of swelling and increases water tension (Ramachandran *et al*., 2004). Pandey *et al*. (1994) reported that with low water availability fungi suffer modification in their cell membranes leading to transport limitations and affecting microbial metabolism. Based on the present results, moisture at 70% seems to result in a compromise among water availability, substrate swelling and oxygen diffusion effect, favouring amylase production by *F. solani* SY7.

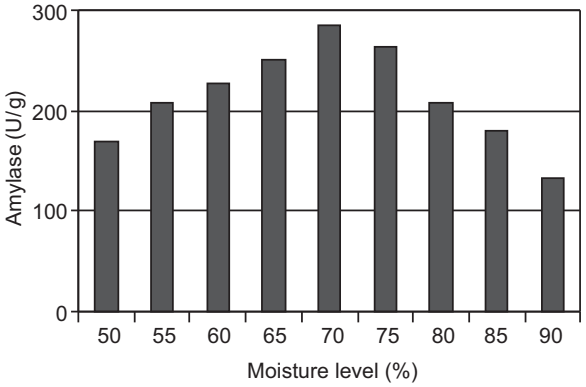

Fig. 4. Effect of moisture (%) on amylase production by *Fusarium solani* SY7.

Conclusion

The present study reveals that *F. solani* SY7 isolate proved to be an efficient producer of α-amylase under improved conditions. Wheat bran could be used as a less expensive substrate for efficient amylase production (141.18 U/g) after 3 days of incubation. The culture conditions can easily be modified to enhance the productivity of the enzyme formation that will facilitate the scale-up processes for biomass production.

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