Research in Biotechnology, 4(1): 31-37, 2013 ISSN: 2229-791X
www.researchinbiotechnology.com

**Regular Article**

# Xylanase Production by *Fusarium Solani* in Solid State Fermentation

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**Xylanase constitute one of the most important groups of enzymes for commercial use. In the present study, production of xylanase was enhanced using a newly isolated *F. solani* SyrN7 strain from infected wheat seeds via solid state fermentation (SSF) using wheat bran as substrate. Optimum pH and temperature for enzyme production were found to be 8 and 25 °C, respectively. The wheat bran to mineral solution ratios were tested. The ratio 1:3 (w/v) yielded the highest xylanase production. Among the different nitrogen sources investigated, yeast extract was the best nitrogen source and gave the highest xylanase activity (1593 U/g). The cultivation systems can easily be modified with the above tested features and enhanced the xylanase yield by 3-fold.**

**Key words:** *Fusarium solani*, solid state fermentation, xylanase activity

Xylanases are hydrolytic enzymes, which catalyze the endohydrolysis of α-1, 4-xylosidic linkages in xylan. The xylan hydrolysis end product has considerable industrial applications in biofuel, artificial sweetener, animal feed production, baking, textile, clarification of fruit juices and coffee extraction (Medel *et al.,* 2002; Li *et al.,* 2010). Besides, xylanases showed increasing interest applications as eco-friendly bleaching agent of pulp in paper industries (Wong *et al.,* 2002).

The use of purified xylan as a substrate to induce xylanase synthesis increases the cost of enzyme production. Therefore, for commercial applications, there have been attempts to develop a bioprocess to produce xylanase in high quantities from simple and inexpensive substrates (Lemos *et al.,* 2001).

Xylanolytic enzymes are produced by a wide variety of microorganisms, among which the filamentous fungi are especially interesting as they secrete these enzymes into the medium and their xylanase activities are much higher than those found in yeast and bacteria (Haltrich *et al.,* 1996; Khan *et al.,* 2003; 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 (Summerell *et al.,* 2001). This ecological habitat of the fungus implies that *Fusarium* would be a useful resource of extracellular enzymes. Several different enzymatic activities were investigated in isolates of *Fusarium* species, including xylanase (Alconada and Martínez, 1994; Jenczmionka and Schafer, 2005; Arabi *et al.,* 2011).

Solid state fermentation (SSF) cultivation offers advantages over liquid cultivation, especially for fungal cultures. This culture system was found to be more economical mainly due to the cheap and abundant availability of agricultural wastes

Received: 15.12.2012; Revised: 4.2.2013; Accepted: 13.2.2013

which can be used as substrates (Pandey *et al.*, 1994). Additionally, these processes are of special economic interest for the countries with abundance of biomass and agroindustrial residues, as these can be used as cheap raw materials (Kumaran *et al.*, 1997). However, the enzyme production is related to the type and concentrations of nutrients and growth conditions (Abdel-Sater and El-Said, 2001). To reach commercial feasibility, enzyme production must be increased by introducing more potent strain and by optimising culture conditions (Haltrich *et al.*, 1996). Thus, since the effects of some basic parameters; pH, temperature, moisture level and nitrogen sources on xylanase production by the fungus *Fusarium solani* has not been investigated so far, a study to this aim has been conducted on the new *F. solani* SyrN7 strain cultured under SSF.

## Materials and methods

**Microorganism:** The strain *Fusarium solani* SyrN7 was described by Arabi *et al.* (2011). It was isolated from infected wheat seeds showing head blight symptoms, and screened among 105 isolates as the best xylanase producer. The strain was grown separately in 9-cm Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) and incubated for 10 days, at (24 ± 1) $^{o}$C in the dark to allow mycelia growth. Stock cultures were maintained on PDA at 4 $^{o}$C.

**Effect of medium pH and incubation temperature:** The influence of initial medium pH on xylanase production was assessed by cultivating the strain in the basal media of pH ranging from 3.0 to 9.0. The effect of temperature was studied by performing the fermentation at different temperatures, 25, 30, 35, 40 and 45.

**Effect of moisture level:** The effect of moisture level on the enzyme production was determined by varying the ratio (w/v) of wheat bran to moistening agent at the ratio of 1:1, 1:2, 1:3, 1.4, 1.5, and 1:6. The moistening agent used was sterile distilled water.

**Solid-state fermentation (SSF) cultivation systems:** Cultivation of fungus was performed in 250 ml Erlenmeyer flask containing 5 g of solid substrate and nutrients (based on 100 ml of liquid medium) plus distilled water. 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. Fresh fungal spores have been used as inocula and 1mL spore suspension (containing around $10^6$ spores/mL) was added to sterilized medium. Flasks were removed after cultivation and the enzyme was extracted by adding distilled water containing 0.1% Triton x 100 to make the volume in flask equivalent to 100 mL. The flasks' contents were stirred for 1.5 hours on a magnetic stirrer. The clear supernatant was obtained by centrifugation (5000 × g for 15 min) followed by filtration (Whatman no 1 paper).

**Nitrogen sources:** The influences of different nitrogen sources as urea, $NaNO_3$, yeast extract, casein, peptone, $KNO_3$, $NH_4Cl$, $NH_4NO_3$ and $(NH_4)_2H_2PO_4$ on xylanase production from *F. solani* SyrN7 were tested. Wheat bran was used as carbon source and incubations were carried out for 4 days.

**Enzyme assay:** Xylanase was assayed by the optimized method described by Bailey *et al.* (1992), using 1% birchwood xylan as the substrate. The xylan solution and the enzyme at appropriate dilution were incubated at 55$^{o}$C for 5 minutes and the reducing sugars were determined by the dinitrosalicylic acid procedure (Miller, 1959), with xylose as the standard. The released xylose was measured spectrophotometrically at 540 nm. One unit (U) of enzyme activity is defined as the amount of enzyme releasing 1 µmol xylose/ml per minute under the described assay conditions.

**Statistical analysis:** The experiments were repeated twice, and all the results represent mean values. Data were subjected to an

analysis of variance (Anonymous, 1996) using the super ANOVA computer package to test for differences in xylanase production among different tests.

**Results and discussion**

Xylanase production by this isolate was observed in the range 3.0-9.0 pH using wheat bran as carbon source (Fig.1). The organism showed a greater xylanase production at a neutral and alkaline pH than at an acidic pH. High xylanase production was (1515 IU/g) at pH 8 and minimum was observed at pH 3 (483 IU/g). When the pH was increased or decreased to other than 8, the production of xylanase gradually decreased. These results might be attributed to the fact that the alkaline pH has inhibitory effect on the mycelium growth and enzyme activity. Moreover, cultivation of fungi in an unfavorable pH value, may favour limited growth rate and xylanase production by reducing accessibility of the hemicellulosic substrate (Poorna and Prema, 2007). In the case of fungi, the majority of researchers have reported an acidic pH to be the most appropriate for maximum enzyme production (Espiner *et al.,* 1992, Shah and Madamwar, 2005). However, Senthikumar *et al.* (2005) obtained maximum xylanase production by *Aspergillus fischeri* Fxn 1 in pH 9 medium, while Sudan and Bajaj (2007) found that Aspergillus niveus RS2 showed a better xylanase production at pH 8.

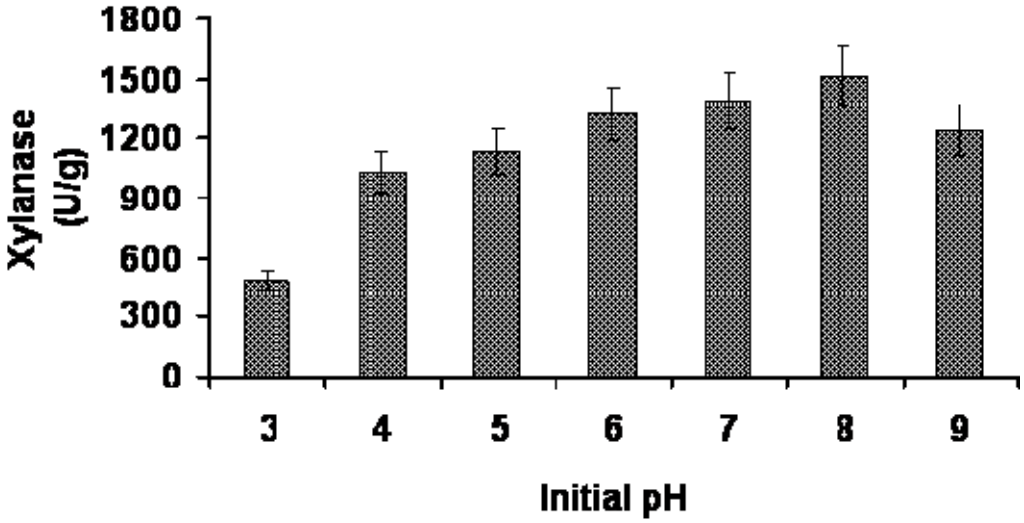


Fig.1: Influence of medium pH on xylanase production by *Fusarium solani* SYN7 grown on wheat bran under solid state fermentation.

Temperature is one of the important parameters that determine the success of SSF system. So, the enzyme activity by *F. solani* SyrN7 was investigated at different temperatures and the results obtained are shown in Figure 2. The production of xylanase was maximum at the ambient temperature 25°C. The optimum temperature for xylanase production is similar to the optimum temperature for the growth of the fungus (Nelson *et al.,* 1983). However, when the temperature increased or decreased from 25 °C, the production of xylanase gradually reduced. The optimum temperature for xylanases from fungal sources has been found to be similar or slightly higher. *Penicillium citrinum* (Considine *et al.,* 1989), *Trichoderma harzianum* (Rezende *et al.,* 2002) presented xylanase with maximum activities at 30 °C.

The moisture content of the substrate was examined by adding external water in the cultivation system using distilled water at different ratio between the substrate,

wheat bran and the amount of water added. As indicated in Figure 3, the xylanase production was optimum using the wheat bran which was moistened with moistening agent in the ratio of 1:3 (initial moisture level 75 %) with the production of 1593 U/g. The fact that the produces maximum xylanase activity at low water ratio offers significant advantage in reducing the risk of contamination, since most bacterial species are unable to grow at reduced moisture level. However, water causes the swelling of the substrate and facilitates good utilization of substrates by the microorganisms. Increasing moisture level is believed to have reduced the porosity of substrate, thus limiting the oxygen transfer into the substrate (Raimbault and Alazard, 1980). Likewise, a lower moisture ratio leads to reduced solubility of the nutrients of the solid substrate, lower degree of swelling and a higher water tension (Ikasari and Mitchell, 1994). The optimum moisture contents for xylanase production by *Trichoderma longibrachiatum* and *Aspergillus tereus* were found to be 55 and 75%, respectively (Guimaraes *et al*., 2006).

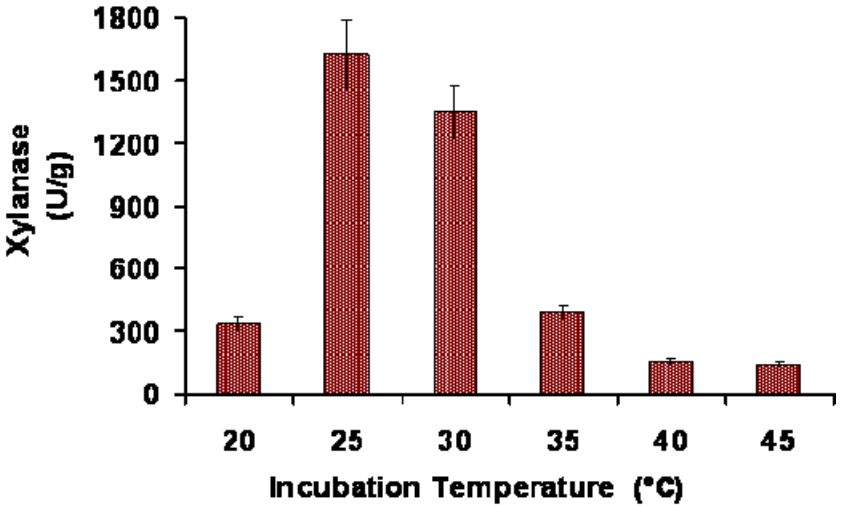


Fig. 2: Influence of temperature incubation on xylanase production by *Fusarium solani* SYN7 grown on wheat bran under solid state fermentation.

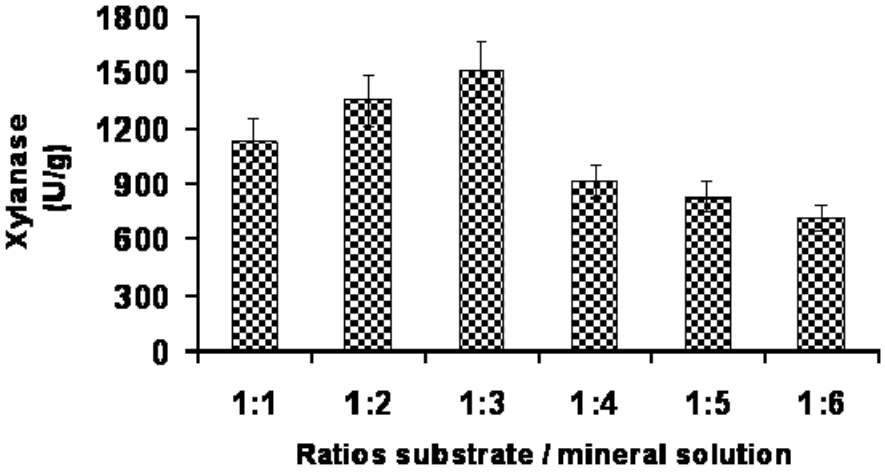


Fig. 3: Effect of substrate to mineral solution ratio on xylanase production by *Fusarium solani* SYN7 grown on wheat bran under solid state fermentation.

The production of primary metabolites by microorganisms is highly influenced by their growth, which is determined by the availability of the nutrients in the substrates. Therefore, it is expected that the improvement of the nutritional value of wheat bran by the supplementation of nitrogen sources will improve the enzyme production by *F. solani* SyrN7. Table 1 shows that yeast extract was the best in enhancing xylanase production and a 3-fold increase in enzyme activity was obtained compared to the control, probably because this complex nitrogen source contains elements that are necessary for the metabolism of fungus (Lemos *et al.*, 2001). Nitrogen sources have a dramatic effect on the production of xylanolytic enzyme by fungi (Kuhad *et al.*, 1998). Our results are in good agreement with those of Lemos *et al.* (2001) and Abdel-Sater and El-Said (2001). Peptone also was effective in inducing the enzyme. The other nitrogen compounds tested were less efficient.

Although quantitative comparison of xylanase activities reported in literature is not always possible because no standard enzyme substrate has been adopted yet, the yield of xylanase productivity from *F. solani* observed in this work were approx 3 folds higher than optimum productivities reported in the literature for some microorganisms grown in SSF (Table 2).

Table 1: Effect different nitrogen sources on xylanase production by *F. solani* SYN7 in SSF after 4 days of incubation.

| Source | Xylanase (U/g) |
|---|---|
| Control | 566 |
| Urea | 112 |
| $NaNO_3$ | 688 |
| Yeast extract | 1593 |
| Casein | 537 |
| Peptone | 1340 |
| $KNO_3$ | 891 |
| $NH_4CL$ | 850 |
| $NH_4NO_3$ | 967 |
| $(NH_4)2H_2PO_4$ | 945 |

LSD: Least Significant Difference at $P < 0.05$

Table 2: Optimum xylanase activities produced by filamentous fungi grown in SSF

| Microorganism | Substrate | Cultivation conditions | Activity IU/g | Reference |
|---|---|---|---|---|
| *Aspergillus niger* 3T5B8 | Wheat bran +0.1 cellobiose | 32°C, 3 d | 100.65 | Couri et al. (2000) |
| *Aspergillus niger* | Wheat bran | 35°C, 3 d | 94 | Widjaja et al.(2009) |
| *Aspergillus niger* USMA11 | Palm cake | 30°C, 7 d | 33.99 | Kheng and Omar (2005) |
| *Chaetomium cellulolytium* | Wheat straw | 37°C, 10 d | 580 | Dubea et al. (1986) |
| *Fusarium solani* | Wheat bran | 25°C, 4 d | 1593 | This work |
| *Penicillium Capsulatum* | Beet pulp + Wheat bran | 30°C, 9 d | 279,9 | Considine et al.(1989) |
| *Trichoderma viride* | Wheat bran +1% sorbitol | 25°C, 5 d | 561 | Simoes et al. (2009) |
| *Trichoderma harzianum* | Sugarcane bagasse | 30°C, 7 d | 82 | Rezende et al. (2002) |

**Conclusion**

The present study reveals that *F. solani* SyrN7 strain is a highly potential and promising microorganism as it produced a high level of xylanase under improved conditions. The basic parameters such as pH, temperature and moisture level found to have exerted a marked influence on the yield of xylanase. Moreover, adding yeast extract resulted in an increased xylanase production (1593 U/g) compared to the

fermentations in which this compound was not used.

**Acknowledgements**

The authors thank the Director General of AECS and the Head of the Molecular Biology and Biotechnology Department for their continuous support throughout this work. Thanks also extended to Dr. A. Aldaoude for critical reading of the manuscript.

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