

Extracellular Xylanase Production by *Fusarium* species in Solid State Fermentation

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Abstract

Fusarium sp. has been shown to be a promising organism for enhanced production of xylanases. In the present study, xylanase production by 21 *Fusarium* sp. isolates (8 *Fusarium culmorum*, 4 *Fusarium solani*, 6 *Fusarium verticillioides* and 3 *Fusarium equiseti*) was evaluated under solid state fermentation (SSF). The fungal isolate *Fusarium solani* SYRN7 was the best xylanase producer among the tested isolates. The effects of some agriculture wastes (like wheat straw, wheat bran, beet pulp and cotton seed cake) and incubation period on xylanase production by *F. solani* were optimized. High xylanase production (1465.8 U/g) was observed in wheat bran after 96 h of incubation. Optimum pH and temperature for xylanase activity were found to be 5 and 50°C, respectively.

Key words: *Fusarium* sp., solid-state fermentation, xylanase

Introduction

To date xylanase has gained increasing attention because of its various biotechnological applications. Endo- β -1, 4-xylanase plays important roles in animal feed, increasing the body weight gains of animals (Medel, *et al.*, 2002). In pulp and paper industry, xylanases are employed in the prebleaching process to reduce the use of toxic chlorine chemicals (Wong *et al.*, 2002). In bread and bakery industry, xylanases are used to increase dough viscosity, bread volume, and shelf life (Romanowska *et al.*, 2003). Other potential applications include the conversion of xylan in wastes from agriculture and food industries into xylose, and the production of fuel and chemical feedstocks (Sunna and Antranikian, 1997). 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). However, to reach commercial feasibility, enzyme production must be increased by introducing a more potent strain and by optimizing culture conditions.

Fusarium is a large genus of filamentous fungi, and most *Fusarium* species are harmless saprobes and relatively abundant members of the soil microbial community (Domsch *et al.*, 1980; Nwanma *et al.*, 1993). This ecological habitat of the fungus implies that *Fusarium* would be a useful resource of extracellular enzymes.

However, information on the ability of xylanase production in *Fusarium* spp. is rarely reported.

Among the processes used for xylanase production, solid state fermentation (SSF) is an attractive one because it presents many advantages, especially for fungal cultivations (Weiland, 1988; Bakri *et al.*, 2003; Arabi *et al.*, 2001).

In SSF, the productivity per reactor volume is much higher compared to that of submerged culture (Haltrich *et al.*, 1996). Also, the operation cost is lower, because simple plant, machinery and energy are required (Poorna and Prema 2007). Many SSF processes for enzyme production, including xylanase, are described in the literature (Pandey, 1994).

The objectives of the present study were (i) to investigate, on artificial growth media, the xylanase production by *Fusarium* sp. Isolates collected from different regions of Syria, and (ii) study the effects of some agricultural wastes on xylanase production by the promising *F. solani* SYRN7 isolate under SSF.

Experimental

Materials and Methods

Fungal isolates. Over several years, more than 105 isolates of *Fusarium* spp. were obtained from wheat seeds showing disease symptoms in different locations of Syria. Seeds were sterilized in 5% sodium hypochlorite (NaOCl) for 5 min. After three washings with sterile

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distilled water, the seeds were transferred onto Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI. USA) with 13 mg/l kanamycin sulphate added after autoclaving and incubated for 10 days, at $23 \pm 1^\circ\text{C}$ in the dark to allow mycelial growth and sporulation. All isolates were identified morphologically according to Nelson *et al.* (1983). In previous studies, different wheat genotypes had been inoculated with 105 fungal isolates, evaluating host-pathogen reactions using the method described by Kiprop *et al.* (2002). Emphasis was placed on selecting isolates that induced differential reactions on specific genotypes (Alazem, 2007), leading to selection of the 21 monosporic isolates (eight belonging to *F. culmorum*, four to *F. solani*, six to *F. verticillioides* and three to *F. equiseti*) used in this study. The *Fusarium* isolates, their host plants, and geographic origin are listed in Table I. The cultures were maintained on silica gel at 4°C until needed.

Xylanase production medium. Enzyme production by the selected isolates was carried out in 250 ml Erlenmeyer flasks containing 5 g of solid substrate and nutrients (based on 100 ml of liquid medium) plus distilled water to adjust the moisture content to 75%. The fermentation medium consisted of: (g/L) $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$ 10; KCl 0.5; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ 0.15, and Yeast extract 5, as a nitrogen source. The influences of different lignocellulosic materials (wheat bran, beet pulp and cotton seed cake) on xylanase production were tested. Fresh fungal spores have been used as inoculums and 1 mL spore suspension (containing around 10^6 spores/mL) was added to sterilized medium and incubated at 30°C . The flasks were removed after cultivation and the enzyme was extracted by adding distilled water containing 0.1% Triton $\times 100$ to make the volume in a flask 100 mL. The flasks' contents were stirred for 1.5 hours on a magnetic stirrer. The clear supernatant was obtained by centrifugation ($5000 \times g$ for 15 min) followed by filtration (Whatman no 1 paper).

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

Effect of temperature and pH on enzyme activity. To determine temperature activity profile for xylanase enzyme, assay was carried out at several temperatures 40, 45, 50, 55, 60, 65, 70, and 75°C at pH 5. The optimum pH was determined by measuring the activity at 50°C using the following buffers: 0.1M Citrate-

Table I
Fusarium isolates, host, location and extracellular xylanase production in solid state fermentation after 5 days of incubation at 30°C

Isolate	Host	Location	Year of collection	Xylanase (U/g)
<i>F. culmorum</i>				
SY1	wheat seeds	north-west	2005	20.3
2	wheat seeds	north-west	2005	96.36
3	wheat seeds	north-west	2005	163.69
4	wheat seeds	north-west	2005	12.16
6	wheat seeds	north-west	2005	131.93
12	wheat seeds	north-west	2005	115.92
13	wheat seeds	north-west	2004	90.64
14	wheat seeds	middle region	2003	19.52
<i>F. verticillioides</i>				
SY9	wheat seeds	north-west	2005	138.72
10	wheat seeds	north-west	2005	19.52
15	wheat seeds	north-west	2004	61.92
17	wheat seeds	middle region	2005	16.56
18	wheat seeds	middle region	2005	129.92
19	wheat seeds	middle region	2004	108.56
21	wheat seeds	middle region	2003	102.3
<i>F. solani</i>				
SY7	wheat root	middle region	2003	908.2
8	wheat seeds	north-west	2004	125.6
11	wheat root	north-west	2005	112.16
20	wheat root	north-west	2004	234.96
<i>F. equiseti</i>				
SY22	wheat seeds	north-west	2005	93.2
23	wheat seeds	north-west	2003	84.64
24	wheat seeds	middle region	2005	122.43
LSD 5%				

LSD: Least Significant Difference at $P < 0.05$

phosphate (pH 4.0–6.0); 0.1 M potassium-phosphate (pH 7.0–8.0) and 0.1M Tris-HCl (pH 9.0).

Statistical analysis. The experiments were repeated twice. Results were subjected to an analysis of variance (Anon., 1996) using the super ANOVA computer package to test for differences in xylanase production among isolates.

Results and Discussion

Table I shows that all the *Fusarium* species were capable of producing xylanase activity but to varying degrees (Table I). Significant differences ($P < 0.05$) in the mean yield values were detected among isolates, with high values being consistently higher in the isolates *F. solani* SYRN7 and SYRN20 with mean value 908.2 U/g and 234.69 U/g, respectively. Low enzyme activities of 12.16 and 16.56 U/g were detected for *F. culmorum* SYRN4 and *F. verticillioides* SYRN17,

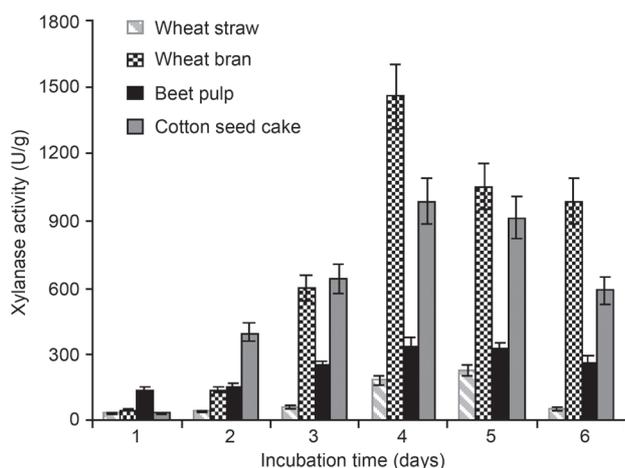


Fig. 1. The effect of lignocellulosic materials (wheat straw, wheat bran, beet pulp, and cotton seed cake) on xylanase production by *Fusarium solani* F7.

respectively (Table I). From this group, *F. solani* SYRN7 isolate was selected for further studies. This isolate was isolated from infected wheat seeds showing disease symptoms, and screened among 105 isolates as the best xylanase producer in SSF culture. The isolate was grown on PDA medium and identified as described above.

Since the cost of the substrate plays a crucial role in the economics of xylanase production process, the expensive substrate (pure xylan) is not suited for larger-scale production processes due to its high cost. Insoluble lignocellulosic materials offer a cost effective substrate for xylanase production (Bakri *et al.*, 2003; Li *et al.*, 2007). To select a suitable carbon source and incubation time for xylanase production, *Fusarium solani* SYRN7 was cultivated in a basal medium containing some lignocellulosic materials wheat straw, wheat bran, beet pulp and cotton seed cake as carbon sources during 6 days. We observed that maximum enzyme activity (1465 U/g) was obtained by using wheat bran after 4 days of incubation (Fig. 1). This indicated that the choice of an appropriate substrate is of great importance for the successful xylanase production. The substrate not only serves as a carbon and energy source, but also provides the necessary inducing compounds for the organism. Wheat bran proved to be the best carbon source followed by cotton seed cake. In some fungi, high xylanase production has been shown to be linked strictly to the ratio of cellulose to xylan of the growth substrate and substrate degradation due to time course or incubation period (Haltrich *et al.*, 1996; Chirstakopoulos *et al.*, 1999; Kang *et al.*, 2004).

Enzyme activity is markedly affected by pH. This is because substrate binding and catalysis are often dependent on charge distribution on both substrate and, in particular, enzyme molecules (Kulkarni *et al.*, 1999). A pH range from 4 to 10 was used to study the effect of pH on xylanase activity and the results are

given in Fig. 2. The favorable pH range for xylanase activity of *Fusarium solani* SYRN7 was between 5.0 and 6.0, with optimum pH at 5.0. A significant drop in enzyme activity was observed below pH 5.0 and above pH 6.0. A sharp decrease of xylanase activity was observed between pH 5.0 (100%) and pH 8.0 (47.16%). The enzyme behaviour clearly indicates that it is more suitable for any application in the pH range of 5.0–6.0. Similar results were observed for other microorganisms. *Aspergillus* sp. (Khanna *et al.*, 1995), *A. oryzae* (Kitamoto *et al.*, 1999), *Fusarium verticillioides* (Saha, 2003), *Penicillium citrinum* (Tanaka *et al.*, 2005) and *Penicillium* sp. AH-30 (Li *et al.*, 2007), presented xylanase with maximum activities at similar pH.

The effect of temperature on the xylanase activity from *Fusarium solani* SYRN7 is shown in Fig. 3. The

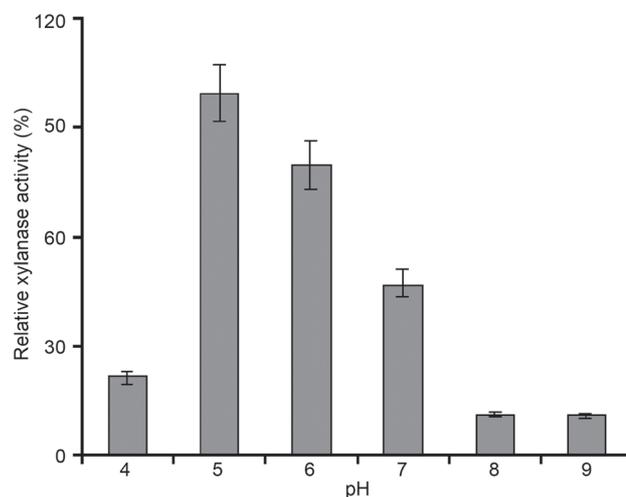


Fig. 2. Optimum pH activity of xylanase produced by *Fusarium solani* F7 grown on wheat bran under solid state culture. Relative activity was determined at 55°C.

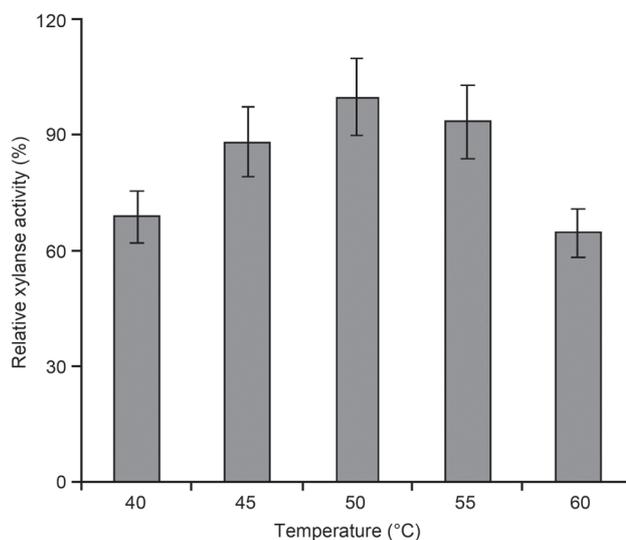


Fig. 3. Optimal temperature of xylanase produced by *Fusarium solani* F7 in solid state culture. Relative activity was determined at pH 5.

optimum temperature was 50°C. When the temperature reached 60°C, relative xylanase activity retained was about 64.75% under the assay conditions used. The optimum temperature for xylanases from fungal sources has been found to be similar or slightly higher. *Penicillium citrinum* (Tanaka et al., 2005), *Penicillium* sp. AH-30 (Li et al., 2007), *Aspergillus sydowii* SBS 45 (Nair et al., 2008) and *Aspergillus niveus* RS2 (Sudan and Bajaj, 2007) presented xylanase with maximum activities at 50°C. *Penicillium purpurogenum* (Belancic et al., 1995), *Aspergillus oryzae* (Kitamoto et al., 1999) and *Aspergillus niger* (Coral et al., 2002) presented xylanase with maximum activities at 60°C.

The present study demonstrated that significant improvement of xylanase production by *F. solani* SYRN7 isolate could be obtained by selective use of nutrients and growth conditions. Since xylan is an expensive substrate for commercial scale xylanase production, the possibility of using wheat bran for xylanase production was investigated. Wheat bran (5% by mass per volume) could be used as a less expensive substrate for efficient xylanase production (1465.8 U/g). This observation is interesting due to the low cost of this carbon source. The *F. solani* SYRN7 isolate proved to be a promising microorganism for xylanase production.

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