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EXPERIMENTAL ARTICLES

Effect of Additional Carbon Source and Moisture Level on Xylanase Production by *Cochliobolus sativus* in Solid Fermentation[1]

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Abstract—The fungus *Cochliobolus sativus* has been shown to be an efficient producer of xylanase from an industrial point of view. The addition of extra carbon sources and the initial moisture content of the solid–state fermentation were found to have a marked influence on the xylanase production by *C. sativus* Cs6 strain. Xylan and starch resulted in an increased xylanase production (1469.4 and 1396.56 U/g, respectively) after 8 days of incubation. Optimal initial moisture content for xylanase production was 80%. The cultivation systems can easily be modified to enhance the productivity of the enzyme formation by *C. sativus* Cs6, which will facilitate the scale up processes for mass production.

Keywords: *Cochliobolus sativus*, xylanase, sugars, solid state fermentation.

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INTRODUCTION

Xylanase has attracted considerable research interest because of their various biotechnological applications [1, 2]. This enzyme has been isolated from diversified range of microorganisms including fungi and bacteria [3]. However, although xylanases from eubacteria and archaebacteria have considerably higher temperature optima and stability than those of fungi, the amount of enzyme produced by these bacteria is comparatively lower than that produced by fungi [4].

The genus *Cochliobolus* has been shown to be an efficient producer of xylanases from an industrial point of view. Several enzymatic activities were investigated in isolates of the fungus *Cochliobolus sativus*, the causal agent of barley spot blotch disease, such as cellulose-hydrolysing enzymes, endo-1,4-β-xylanase and endopolygalacturonase [5, 6].

Among processes used for enzyme production, solid sate fermentation (**SSF**) is an attractive one because its presents many advantages, especially for fungal cultivations [7]. In SSF, the productivity per reactor volume is much higher compared to that of submerged culture [8]. Also, the operation cost is lower, because simple plant, machinery and energy are required [9]. Many SSF processes for enzyme production, including xylanase, are described in the literature [10].

Enzyme production is related to the type and concentrations of carbon sources [11]. Recently, a strain *C. sativus* Cs6 was described as a good producer of xylanase in SSF using different agricultural wastes as the main nitrogen sources [12]. This work complements a previous one that investigated the effects of additional carbon sources and moisture level on xylanase production by the newly *C. sativus* strain Cs6 under SSF.

MATERIALS AND METHODS

Fungal strain. The fungal strain of *C. sativus* Caused in this work was described by Arabi and Jawhar [13]. It was isolated from infected barley leaves showing spot blotch symptoms, and screened among 117 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 22 ± 1°C in the dark to allow mycelia growth.

Liquid culture. Enzyme production by the selected strain was carried out in 250 ml Erlenmoyer flasks containing 5 g of wheat straw and nutrients (based on 100 ml of liquid medium) plus distilled water to adjust the moisture content to 80%. The fermentation medium consisted of: (g/L) $Na_2HPO_4 \cdot 2H_2O$ 10; KCl 0.5; $MgSO_4 \cdot 7H_2O$ 0.15, and $NaNO_3$ 5, as a nitrogen source. The influences of the incubation time on xylanase production were tested (Fig. 1). The pH was adjusted to 4.5 before sterilization. This pH value was found to be the optimal one for xylanase production in our experiments (data not included). Fresh fungal spores have been used as inoculum and 1 mL spore suspension (containing around 10^6 spores/mL) was added to sterilized medium and incubated at 30°C.

Flasks were removed after cultivation and the enzyme was extracted by adding distilled water con-

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taining 0.1% Triton × 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).

Carbon sources. Xylanase was produced by *C. sativus* Cs6 in the basal medium supplemented with sugars (2%) of (glucose, sucrose, maltose, xylose, dextrose, starch, mannitol and xylan. Lignocellulosic substrate (wheat straw) was used as the carbon source in fermentation medium. The effects of different concentrations of $NaNO_3$ between 0.1 and 0.8% were tested on xylanase production by *C. sativus*.

In order to understand the effect of water availability on xylanase production, the changing the wheat straw to mineral solutions was studies. Seven different ratios were tested. It was taken into consideration that the concentration of soluble medium ingredients was not changed. In all cases wheat straw was used as the solid substrate. Sterile distilled water was used as the moistening agent.

Enzyme assay. Xylanase activity was assayed by the optimized method described by Bailey et al. [14] 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 [15], 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 μmol xylose/ml per minute under the described assay conditions.

The experiments were repeated twice, and all the results represent mean values. Statistical analyses were performed using the STAT-ITCF program [16] to test for differences in xylanase production among different tests.

RESULTS AND DISCUSSION

Figure 1 shows that the highest xylanase production was obtained on wheat straw cultures after 8 days of incubation, thereafter the enzyme activity declined. Similar observations were reported for the production of pectinases and cellulose by fungi on SSF [17]. However, when the time was increased or decreased to other than 8 days, the production of xylanase gradually decreased. This might be due to the fact that cultivation of fungi for an extra time could affect the pH of medium, which may favour limited growth rate and xylanase production by reducing accessibility of the hemicellulosic substrate [1].

When a number of carbon sources were tested using xylan addition to wheat straw as a substrate, the results (Table 1) showed that the fungus was able to produce a high activity of xylanase (1469.4 U/g after 8 days). This can be attributed to that xylan and its derivatives play an important role as inducer of xylanase formation [8]. The results shown in Table 1 indicate the minimize catabolic repression of simple sugar in SSF with wheat straw as substrate. Some authors have suggested that the absence of catabolic repression in SFF systems is due to several factors collectively, including the slow and low processes of diffusion in solid state cultures due to the low water activity [14]. The results are in agreement with the results of MacCabe et al. [18] on *Aspergillus nidulans*. Haltrich et al.[8] suggested that low molecular mass degradation products of xylan and cellulose hydrolysis penetrate into the cells and induce the production of hydrolytic enzymes. However, other sugars, such as starch and xylose, were also found capable of inducing xylanase (1396.65 and 1116.75, respectively).

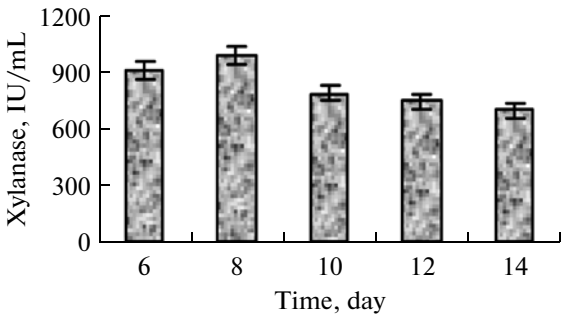

Fig. 1. Effect of time on xylanase recovery by *C. sativus* when wheat straw used a substrate in solid-state fermentation.

The results showed that the ratios 1 : 4 and 1 : 5 (initial moisture level above 80%) yielded the highest xylanase activities (Fig. 2a). This could be attributed to the faster growth of the fungus at higher moisture content and the subsequent early initiation of enzyme production. However, low moisture levels is known to decrease the metabolic and enzymatic activity probably due to reduced solubility of nutrients from the solid substrate, low substrate swelling and higher water tension [19]. The optimum moisture contents for xyla-

Table 1. Effect of different carbon sources on xylanase production by the fungus *C. sativus*

Source (2%)	Xylanase activity (U/g)
Control	1114.5c
Maltose	cd1087.65
Sucrose	d1037.25
Dexstrose	c1100.4
Manitol	cd1077.75
Glucose	d1.044.15
Xylose	c1116.75
Xylan	a1469.4
Starch	b1396.65

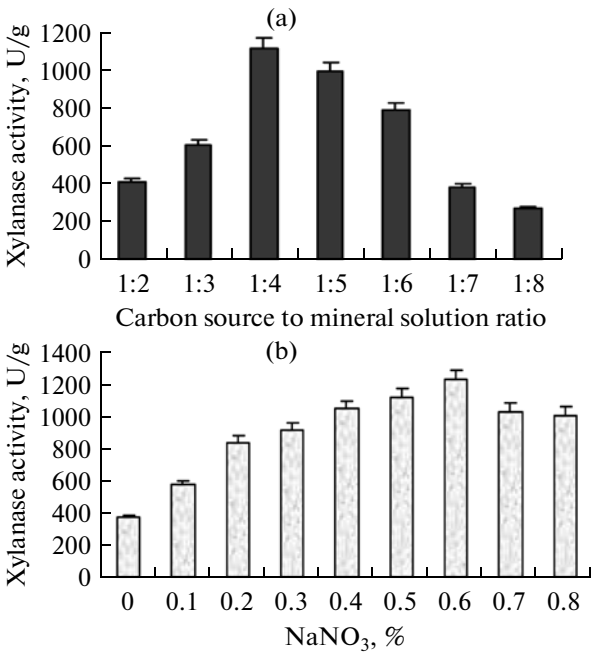

Fig. 2. Effects of carbon sources to mineral solution ratio (a), and different concentration of $NaNO_3$ (b) on xylanase production by *C. sativus* in solid-state fermentation.

nase production by *Trichoderma longibrachiatum* and *Aspergillus tereus* were 55 and 75% [20].

The results also demonstrated that xylanase production by *C. sativus* was very much dependent on $NaNO_3$, and that the optimum concentration was 0.6% (Fig. 2b). However, when the $NaNO_3$ was increased or decreased to other than 0.6%, the production of xylanase gradually decreased. Our results are in good agreement with those of Kuhad et al. [21]. Inorganic nitrogen sources like sodium nitrate have been reported to reduce sporulation by decreasing conidiation level, and this reduction reduces the extracellular protease secretion by *Aspergillus niger* in SSF [22]. The increased amount of $NaNo_3$ results in a reduction in sporulation of *C. sativus*, thus reducing the xylanase activity.

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

The present study demonstrated that significant improvement of xylanase production *C. sativus* Cs6 strain could be obtained by selective use of nutrients and growth conditions. Moisture content and addition of xylan were found to have exerted a marked influence on the yield of xylanase. Moreover, combinations of sodium nitrate with wheat straw resulted in an increased xylanase production compared to the fermentations in which these compounds were not used. As a consequence, *C. sativus* Cs6 strain could be considered as a promising as it produced a high level of xylanase under SSF improved conditions.

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

Microorganism	Substrate	Cultivation conditions	Activity (IU/g of substrate)	Reference
Aspergillus niger 3T5B8	Wheat bran cellobiose+	32°C, 3d	101	Couri et al. 2000 [23]
Aspergillus niger USMA11	Palm cake	30°C. 7d	34	Kheng and Onar, 2005 [24]
Chaetomium cellulolytium ATCC32319	Wheat straw	37°C, 10d	580	Dubeau et al. 1986 [25]
Cochliobolus sativus	**Wheat straw**	30°C, 8d	**1468**	**This work**
Penicillium capsulatum	Beet pulp + wheat bran	30°C, 9d	280	Considine et al. 1989 [26]

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