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Improvement of Xylanase Production by *Cochliobolus sativus* in Submerged Culture

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Summary

The xylanase production by a new *Cochliobolus sativus* Cs5 strain was improved under submerged fermentation. The xylanase was induced by xylan and repressed by glucose, sucrose, maltose, xylose, starch and cellulose. Highest enzyme production (98.25 IU/mL) was recorded when wheat straw (4 % by mass per volume) was used as a carbon source after 120 h of incubation. $NaNO_3$ increased xylanase production 5.4-fold as compared to the control. Optimum initial pH was found to be 4.5 to 5. The *C. sativus* Cs5 strain grown under submerged culture in a simple medium proved to be a promising microorganism for xylanase production.

Key words: xylanase, *Cochliobolus sativus*, waste utilization, submerged fermentation

Introduction

Xylanase has gained increasing attention because of its various biotechnological applications (*1*,*2*). To reach commercial feasibility, enzyme production must be increased by introducing a more potent strain and by optimising culture conditions (*3*,*4*).

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 (*4*).

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 (*3*). Filamentous fungi, particularly *Cochliobolus* sp., are useful producers of xylanase because they are capable of producing high levels of extracellular enzymes and can be cultivated very easily. However, several enzymatic activities have been investigated in the 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*). The enzyme production is related to the type and concentrations of nutrients and growth conditions (*7*). Thus, since the effect of carbon and nitrogen sources on xylanase production by the fungus *Cochliobolus sativus* has not been investigated so far, a study to this aim has been conducted on the new *C. sativus* Cs5 strain cultured under submerged fermentation.

Materials and Methods

Fungal strain

The strain *C. sativus* Cs5 was described by Arabi and Jawhar (*8*). 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 the growth of mycelia. Stock cultures were maintained on PDA at 4 °C.

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Effect of pH

To study the effect of pH, the xylanase activity was measured at various pH values ranging from 4 to 8. The pH of the reaction mixture was adjusted using 1 M NaOH or 1 M HCl before sterilization.

Liquid culture

Xylanase production by the new *C. sativus* Cs5 strain was carried out in Erlenmeyer flasks (250 mL) containing 50 mL of basal culture medium (in g/L): yeast extract 5.0, $Na_2HPO_4{\cdot}2H_2O$ 10.0, KCl 0.5 and $MgSO_4{\cdot}7H_2O$ 0.15. Fresh fungal spores were used as inoculums and 1-mL spore suspension (containing around 10^6 spores/mL) was added to the sterilized medium and incubated at 30 °C for 5 days in a rotary shaker (120 rpm).

Carbon sources

Xylanase was produced by *C. sativus* Cs5 in the basal medium supplemented with sugars (glucose, sucrose, maltose, xylose, starch, cellulose and xylan), and different agricultural and industrial wastes (wheat straw, corn seed powder, barley straw, corn cob hulls, olive pulp, cotton seed, orange peel powder and sawdust).

Nitrogen sources

Yeast extract was replaced with 0.5 % of peptone, urea, NH_4NO_3, $NaNO_3$, NH_4Cl, KNO_3 and $(NH_4)_2SO_4$. Incubations were carried out at 30 °C for 5 days on a rotary shaker (120 rpm). Wheat straw at 4 % concentration was used as carbon source.

Enzyme assay

The culture medium was centrifuged at 6259×*g* for 15 min and the supernatant was used as a source for enzyme sample. Xylanase activity was measured with the optimized method described by Bailey *et al.* (*9*), using 1 % birchwood xylan as substrate. The solution of xylan and the enzyme at appropriate dilution were incubated at 55 °C for 5 min and the reducing sugars were determined by the dinitrosalicylic acid method described by Miller (*10*), with xylose as standard. The released xylose was measured spectrophotometrically at 540 nm. One unit of xylanase is defined as the amount of enzyme required to release 1 μmol of reducing sugar as xylose equivalent per min under the above assay conditions. All experiments were repeated twice.

Results and Discussion

Effect of different pH values

The results showed that xylanase production by *C. sativus* was very much dependent on pH, and the optimum initial pH was between pH=4.5 and 5 (Fig. 1). However, when the pH was increased or decreased to values other than 4.5, the production of xylanase gradually decreased. This might be due to the fact that alkaline pH has inhibitory effect on the growth of *C. sativus* and enzyme production. The initial pH influences the transport of several species of enzyme across the cell membrane. In addition, cultivation of fungi at an unfavourable pH value may favour limited growth rate and xylanase production by reducing accessibility of the hemicellulosic substrate (*11*,*12*).

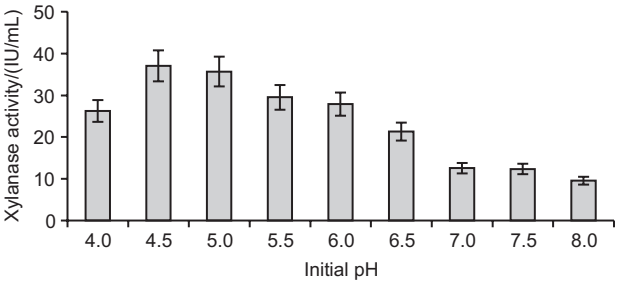

Fig. 1. Effect of different initial pH values on xylanase production by *C. sativus*

Effect of carbon sources on xylanase production

The results shown in Table 1 indicate the inducible nature of enzyme production by *C. sativus*. While a very low xylanase production of 0.26, 0.29, 0.52, 0.71 and 2.43 IU/mL was detected in the medium containing glucose, sucrose, maltose, xylose, starch and cellulose, respectively, in the medium containing xylan, the amount of xylanase reached a level of 34.19 IU/mL after 120 h. The results are in agreement with the results of MacCabe *et al.* (*13*) on *Aspergillus nidulans*. Haltrich *et al.* (*3*) suggested that low molecular mass degradation products of xylan and cellulose hydrolysis penetrate into the cells and induce the production of hydrolytic enzymes. Ghosh *et al.* (*14*) reported that xylose, the ultimate breakdown product of xylan, serves as a good inducer of this enzyme. However, other sugars, such as glucose and CM-cellulose, were found completely incapable of inducing xylanase (<0.26 IU/mL).

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

Source (1%)	Xylanase activity/(IU/mL)
Glucose	0.26
Sucrose	0.29
Maltose	0.52
Xylose	0.48
Starch	0.71
Cellulose	2.43
Xylan	34.19

In this respect, maximum xylanase activity (52.81 IU/mL) was obtained in the medium containing 1 % wheat straw after 120 h of incubation in comparison with the other agricultural and industrial wastes (Table 2). In order to determine the best amount of wheat straw for xylanase production, different concentrations (1–5 % by mass per volume) were tested. The results showed that the highest yield of xylanase was 98.25 IU/mL with 4 % wheat straw (Fig. 2). Increasing the concentration for more than 5 % resulted in a significant decrease in xylanase activity. This might be attributed to the fact that high concentration of substrate led to the increase in medium viscosity, which influenced the mix-

Table 2. Effect of different agricultural and industrial wastes on xylanase production by *C. sativus*

Source	Xylanase activity/(IU/mL)
Wheat straw	52.81
Corn seed powder	3.12
Barley straw	27.75
Wheat bran	10.67
Corn cob bulls	42.20
Olive pulp	1.58
Cotton seed	5.75
Orange peel powder	1.92
Sawdust	0.51

Fig. 2. Effect of different concentrations of wheat straw on xylanase production by *C. sativus*

ture medium components and oxygen transfer. Similar results were obtained by other researchers by using high concentrations of lignocellulosic materials as substrates for enzyme production (*15*,*16*).

Effect of different nitrogen sources

Among the various inorganic and organic nitrogen sources tested, 0.5 % of $NaNO_3$ was the best in stimulating xylanase production by *C. sativus* and a 5.4-fold increase in enzyme activity was obtained compared to the control (Fig. 3). Nitrogen sources have a dramatic effect on the production of xylanolytic enzyme by fungi (*15*). Our results are in good agreement with those of Lemos *et al.* (*4*), and Abdel-Sater and El-Said (*7*). Potassium nitrate, yeast extract and peptone were also effective in inducing the enzyme. The other nitrogen compounds tested were less efficient.

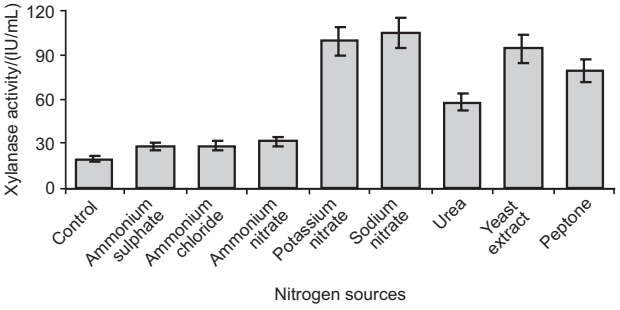

Fig. 3. Effect of different nitrogen sources on xylanase production by *C. sativus*. Wheat straw at 4 % concentration was used as carbon source

Conclusions

The results obtained from submerged culture indicate that significant improvement of xylanase production by *C. sativus* Cs5 strain 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 agricultural residues for xylanase production was investigated. Wheat straw (4 % by mass per volume) could be used as a less expensive substrate for efficient xylanase production (98.25 IU/mL). This observation is interesting due to the low cost of these carbon sources.

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