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Xylanase Production from *Trichoderma harzianum 1073 D3* with Alternative Carbon and Nitrogen Sources

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Summary

The effect of some natural wastes (orange pomace, orange peel, lemon pomace, lemon peel, apple pomace, pear peel, banana peel, melon peel and hazelnut shell) on the production of xylanase from *Trichoderma harzianum 1073 D3* has been studied and maximum activity has been observed on melon peel (26.5 U/mg of protein) followed by apple pomace and hazelnut shell. Also, molasses could be used as an additional carbon source as it decreased the production time approximately by 50 %. Finally, potential alternatives of organic nitrogen source (cotton leaf and soybean residue wastes) were analyzed and it was concluded that peptone could be replaced with these residues especially when economics of the process is the major objective.

Key words: xylanase, *Trichoderma harzianum*, waste utilization, fruit wastes, molasses

Introduction

Xylans are major structural polysaccharides in plants. They represent up to 30 % of the dry weight of the cell walls of monocotyledons and constitute a minor component of dicotyledons (*1*). Xylans belong to the group of complex structural polymers collectively referred to as hemicelluloses (*2*). Xylanases are important in the bioconversion of hemicelluloses into their constituent sugars.

Lignocellulosic materials are widespread in nature and are renewable resources, therefore making use of them in various industries is of great importance. In the last decade, production of xylanase enzymes has attracted the attention of many researchers as these enzymes are essential for the degradation of plant biomass. Xylanases have potential applications in the pulp and paper, food, feed and beverage industries (*3–6*).

As is the case with all types of wastes, waste minimization and reuse of solid wastes is always preferable over waste treatment and/or waste stabilization. Where applicable, reuse and recycling greatly minimize the volume of solid waste to be disposed. Besides, disposing only of the portion of the solid waste that cannot be reused or recycled not only minimizes waste disposal expenses, but also contributes to the prevention of pollution and sustainable use of natural resources.

For commercial applications, xylanases should ideally be produced quickly and in large quantities from simple and inexpensive substrates. Natural xylan sources such as agricultural and forestry wastes, paper industry wastes and various fruit wastes are potential raw materials for xylanase production. Among these, food industry wastes contain high amount of xylan, as it is one of the main polymers in the plant cell wall. These wastes are potential raw material for xylanase production and as xylanases have a wide range of application, economical production of these enzymes is of great importance.

In this study, our objective was to produce xylanase enzyme on wastes from *T. harzianum 1073 D3* in the most suitable way, therefore the effect of additional carbon and nitrogen sources in the production media was

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investigated. In this respect, xylanase production on some natural wastes in the absence of xylan in the production media was investigated in order to minimize raw material costs. Besides, agricultural and domestic wastes can be converted into useful products.

Materials and Methods

Microbial strain

In this study, *T. harzianum 1073 D3* from Marmara Research Center was used for the production of the xylanase enzyme. Stock cultures were maintained on potato dextrose agar at 4 °C.

Medium

Medium described by Kim *et al*. (*7*) was used with some modifications for growth and enzyme production. Medium contained (in g/L); proteous peptone 0.5, urea 0.3, KH_2PO_4 0.2, $CaCl_2$ 0.3, Tween-80 0.2, $(NH_4)_2SO_4$ 1.4, $MgSO_4{\cdot}7H_2O$ 0.3 and 1 % of birchwood xylan (Sigma) as carbon source. Medium, at pH=5, was sterilized in autoclave at 121 °C, 1.5 atm for 15 min and trace element solution at 0.1 % concentration was added. The trace element solution contained (in g/L): $FeSO_4$ 0.05, $ZnSO_4{\cdot}7H_2O$ 0.014, $CoCl_2$ 0.02, $MnSO_4$ 0.016.

Inoculation

One-mL spore suspensions containing $15{\cdot}10^6$ spores were inoculated into 100-mL growth medium. Incubations were carried out at 30 °C in 7 days on a rotary shaker (150 rpm).

Enzyme activity

The enzyme activity was measured similarly to the method described by Khanna and Gauri (*8*) using 0.1 M sodium acetate buffer at pH=5. The culture filtrate was centrifuged at 7200 rpm for 15 min and used as the enzyme sample. One mL of 1 % xylan solution and 0.5 mL of enzyme sample were mixed in the reaction tubes and the mixture was incubated for 30 min at 37 °C prior to centrifugation. The amount of reducing sugar in the reaction tubes was measured using the dinitrosalicylic acid (DNS) method described by Miller (*9*). The absorbance was read at 550 nm using Jenway, 105 UV/VIS spectrophotometer.

The amount of reducing sugar was calculated from the standard curve based on the equivalent amount of glucose. One unit of xylanase activity was described as the amount of enzyme producing 1 μmol of reducing sugar in 1-mL medium in 1 min under standard test conditions. The amount of protein was determined by Lowry method (*10*) and calculated from the standard curve based on bovine serum albumin.

Alternative carbon sources

Xylan was replaced with 2.5 % of orange pomace, orange peel, lemon pomace, lemon peel, apple pomace, pear peel, banana peel, melon peel and hazelnut shell separately after dehydration. Then, xylan in the production medium was replaced with 1 % molasses, which is one of the major wastes of sugar industry. Finally, production medium was prepared by adding 0.2 % molasses containing 1 % of xylan and xylanase specific activities were determined at 5, 6, 7, 8, 9 and 10 days.

Alternative nitrogen sources

Peptone was replaced with: 0.1 % cotton leaf residue; 0.1 % soybean residue; and 0.05 % cotton leaf residue and 0.05 % soybean residue mixture. Incubations were carried out at 30 °C in 7 days on a rotary shaker (150 rpm).

Results and Discussion

Xylanases are produced by many different types of microorganisms such as *Trichoderma*, *Bacillus*, *Aspergillus*, *Penicillium*, *Schizophyllum*, *Aureobasidium* and *Talaromyces* spp. In similar studies it was concluded that *Trichoderma* spp. are effective producers of xylanase (*11–16*).

The majority of the previous studies on xylanases concentrate mainly on enzyme production and characterization rather than economics of the process (*15,17–22*). However, economics of the process should also be taken into account especially for commercial applications. Indeed, the use of carbon sources such as purified xylan would be too expensive to produce xylanolytic enzymes. Therefore, more research should be carried out on economical production of xylanases.

In this respect, different wastes (orange pomace, orange peel, lemon pomace, lemon peel, apple pomace, pear peel, banana peel, melon peel and hazelnut shell) were tested and maximum activity was observed on melon peel followed by apple pomace and hazelnut shell (Fig. 1). There is a limited number of studies on this subject in literature, among which the most similar one is carried out with the banana plant residue (*23*). Also apple pomace was used for xylanase production

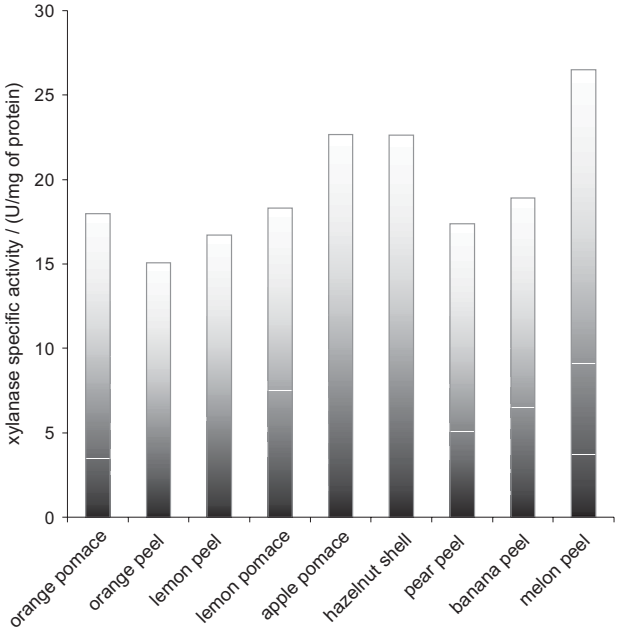

Fig. 1. Effect of different xylan sources on xylanase production. Incubations were carried out at 30 °C for 7 days on a rotary shaker (150 rpm). Results are the averages of three studies

from *Chaetomium globosum* and *Aspergillus niger* in several media containing combinations of different carbon sources (*24*). In a study carried out by Villas-Boas *et al*. (*25*), xylanase production on apple pomace from *Candida utilis* was found to be comparatively low. In addition, apple pomace was used in the production of some other enzymes as well as xylanase (*26,27*).

In a study carried out with *Streptomyces actuosus A-151*, it was concluded that the addition of orange peel to the culture medium enhances the production efficiency of xylanase (*28*). But our experimental results reveal that orange pomace was more efficient when compared to orange peel. In another study, xylanase was produced on banana agricultural waste from *Pleurotus* sp. (*29*).

On the other hand, molasses is the main by-product in the sugar industry, which can be used as an economical carbon source for the fermentation process, and is composed of inorganic and organic nutrients and vitamins as well as carbohydrates. For that reason, xylan in the production medium was replaced with molasses, which contains high amount of sucrose, in order to investigate the effect of molasses on xylanase production, and it was observed that xylanase activity was 27.9 U/mg of protein. Next, molasses was added to the medium containing 1 % of xylan as an additional carbon source. Xylanase specific activity was determined on days 5–10 and it was observed that the activity reached its maximum value (45.2 U/mg of protein) at the end of day 7 (Fig. 2). However, for the medium containing only xylan, activity was 46.3 U/mg of protein on the 13th day. In a similar study, in which the only carbon source in the medium was xylan, the maximum activity was on the 13th day (*30*). Therefore, utilizing molasses for the production of xylanase is economical, needs shorter production time and is environmentally sound because it reduces the amount of waste.

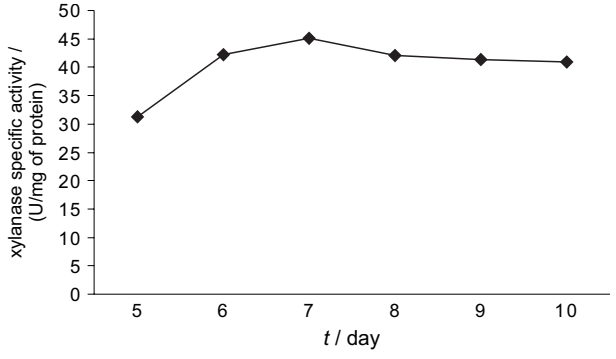

Fig. 2. Effect of incubation time on xylanase production. Incubations were carried out at 30 °C for 10 days on a rotary shaker (150 rpm). Results are the averages of three studies

The major organic nitrogen source used in xylanase production from *T. harzianum 1073 D3* was peptone, which was one of the basic constituents in the production media. However, since peptone is a relatively expensive material, more economical nitrogen sources as potential alternatives to peptone were investigated. These alternatives, namely cotton leaf and soybean residue, were added to the production medium both separately and together and xylanase specific activities were determined. When the results presented in Table 1 were investigated, it can be seen that the activities of these alternatives were lower than the activity of peptone. However, as those nitrogen sources are economical compared to peptone, they can be reasonable alternatives for the production of xylanase in industrial scale, where peptone is not preferred due to its high cost. In a study carried out by Bakir *et al*. (*31*) soybean bagasse was used as both the nitrogen and carbon source in the enzyme production medium and xylanase was produced by *Rhizopus oryzae*.

Table 1. Effect of cotton leaf and soybean residues on xylanase synthesis

	Xylanase specific activity/(U/mg of protein)
Medium containing peptone	44.9
Medium containing cotton leaf residue	22.1
Medium containing soybean residue	21.8
Medium containing cotton leaf and soybean residue	23.4

Conclusion

It can be concluded that among the studied wastes that would replace xylan, melon peel has the maximum activity followed by apple pomace and hazelnut shell. These waste materials constitute a renewable resource and can serve as an abundant and inexpensive carbon source. It was also observed that peptone can be replaced with cotton leaf and soybean residues where appropriate. This observation is interesting due to the low cost of these nitrogen sources. As a result, the use of the above-mentioned wastes in the production of xylanase would decrease the cost of production in an environmentally sound manner.

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Proizvodnja ksilanaze s pomoću *Trichoderma harzianum* koristeći različite izvore ugljika i dušika

Sažetak

Ispitan je utjecaj nekih prirodnih otpadaka (kaše naranče, limuna i jabuke, kore naranče, limuna, kruške, banane i dinje te ljuske lješnjaka) na proizvodnju ksilanaze s pomoću *Trichoderma harzianum.* Maksimalna aktivnost opažena je korištenjem kore od dinje (26,5 U/mg proteina), a nešto slabija postignuta je s kašom od jabuka i ljuskom od lješnjaka. Kao dodatni izvor ugljika može se koristiti i melasa, čime se skraćuje vrijeme proizvodnje za približno 50 %. Analizirani su potencijalni alternativni izvori organskog dušika (lišće pamučike i otpaci soje) pa se može zaključiti da se peptoni mogu nadomjestiti tim ostacima, osobito ako se nastoji postići ekonomičnost procesa.