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PURIFICATION AND CHARACTERIZATION OF XYLANASE FROM *TRICHODERMA* CULTURE USING SSF

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ABSTRACT

Saw dust, rice husk and bagasse were studied as substrates for xylanase production from a *Trichoderma* culture using solid state fermentations (SSF). The characterization of the enzyme was perfomed for xylanase activity. It was found that out of the three substrates used saw dust acted as the best substrate for production of xylanase. The xylanase extracted was found to have an optimal pH 5 and optimal temperature 50^{o}C.

KEY WORDS: Xylanase, *Trichoderma* culture, SSF

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INTRODUCTION

This enzyme is produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insects, seeds, etc. but the principle commercial source is filamentous fungi. 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. In general, the level of xylanase in fungal culture is typically much higher than those from yeasts or bacteria[12]. *Trichoderma* sp. is a good candidate organism for the xylanase production at industrial scale, because, they are non-pathogenic, capable of producing high levels of extracellular enzymes and can be cultivated very easily. Compared with the submerged fermentation, SSF possesses several advantages such as higher fermentation productivity; higher end-concentration of products, higher product stability, lower catabolic repression, cultivation of microorganisms specialized for water-insoluble substrates or mixed cultivation of various fungi, and lower demand of sterility due to low water activity used in SSF[7]**.** Xylanase is also now used in animal feed industry to reduce viscosity of the feed and increases the absorption and digestibility of nutrients[2] and the food industry to improve the dough properties and baking quality of bread and other baked goods[3] as well as to clarification of juices, improvement in consistency of beer, extraction of coffee, extraction of plant oils and starch [4, 5, 11].

MATERIALS & METHODS

In the present study xylanase production and extraction was attempted using *Trichoderma* culture with SSF as the mode of production.

Isolation of Trichoderma and preparation of seed culture

Trichoderma sp. was isolated from garden soil using serial dilution technique by preparing aliquots of up to 10^{-5} dilution from 1g of soil sample and plating them on Czapek Dox agar. The obtained colonies were then observed morphologically and microscopically to screen for *Trichoderma* culture obtained colonies were then used to prepare seed culture in Czapek Dox liquid medium.

Solid state fermentation

Saw dust, rice husk and bagasse were used for SSF. The substrates were sterilized by autoclaving at 15 psi for about 1 h and dried in hot air oven overnight at 57^{o}C to remove moisture. About 1 ml of seed culture was added then inoculated onto the solid substrates (about 5 g) each taken in separate Petri plates. The plates were maintained undisturbed for 6–10 days growth.

Extraction and purification of xylanase

The culture from each plate was collected and ground well in 100 ml of 10 mM Tris HCl using mortar and pestle and the obtained mixture was centrifuged at 10,000 rpm for 10 min. The supernatant obtained was used for enzyme extraction. The proteins in the extract were then precipitated using the ammonium sulfate precipitation method at 70% saturation and the precipitate resuspended in 50 mM Tris HCl buffer (pH 5) and centrifuge it. This was then dialysed using Cellulose acetate membrane at 4^{o}C overnight. The dialysed solution was then fractionated by ion-exchange chromatography through a DEAE column using linear gradient solutions of Tris buffer containing 25–150 mM NaCl as eluent. The purified enzyme was run through SDS-PAGE to determine molecular weight.

Enzyme assay and characterization[2]

The extract samples from cultures grown on three different substrates were assayed to determine total enzyme activity and specific activity at each step of purification, in crude form (direct extract), partially purified form (after dialysis) and highly purified form (after ion-

exchange chromatography). The Assays were conducted using xylan as the substrate and glucose the end product detected by DNS method. The obtained activities for extract from each culture were compared.

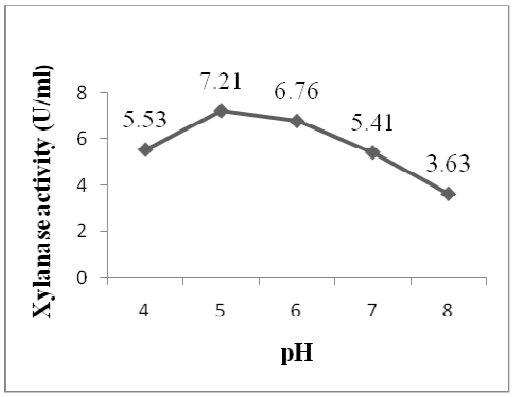
RESULTS & DISCUSSION

The results of total enzyme activities and protein content are summarized in table.

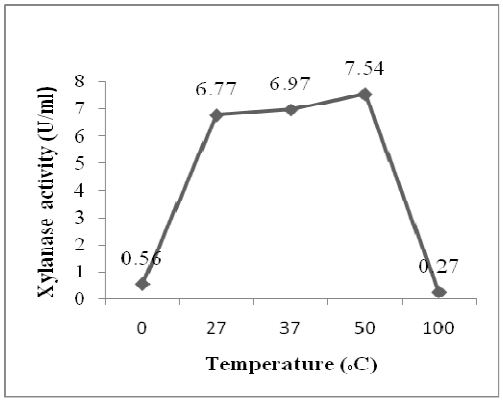
Table
Comparison of total enzyme activities for extracts from Trichoderma cultures grown on three different substrates

Total enzyme activity (U/ml)	Saw dust	Rice husk	Bagasse
Crude	6.11	6.13	5.00
Partially purified	6.9	6.55	4.75
Highly purified	7.23	6.76	6.4
Protein content (mg/ml)	0.02	0.009	0.006

Characterization experiments of the enzyme in its highly purified form showed that the optimal pH for the enzyme is 5 and the optimal temperature is 50°C. The characterization results are summarized in graph 1 and 2. The activity of partially purified β-1, 4xylanase was optimal at pH 5.5 and 40ºC in the study[10]. *Penicillium expansum*, *P. chrysogenum*[8] and *Aspergillus fischeri* Fxn1[3] have also presented xylanases with maximum activities at similar pH, optimal pH 5 for a xylanase produced by *A. oryzae*[9], optimal pH 5 for a xylanase of *A. flavipes*[13] were observed. The xylanases of fungal origin usually show optimal activity around 50ºC, being inactivated at 65ºC[6], optimal temperature 60ºC for the xylanase produced by *A. oryzae*[9], whereas an optimal temperature 55 ºC for a xylanase produced by *A. flavipes* [13].



Graph 1



Graph 2

The molecular weight of the extracted xylanase from *Trichoderma* culture determined using SDS-PAGE was 20 kDa. The molecular weight determined for the xylanase in this study is consistent with the several low molecular weight xylanases (molecular weights, 20,000–22,000 Da) that have been studied in earlier works[1, 14].

CONCLUSION

Saw dust, rice husk and bagasse, saw dust proved to be the best substrate for the production of xylanase using *Trichoderma* culture. The results of the present study indicate the ability of SSF for production of xylanase using *Trichoderma* culture as the fermenter organism and organic waste products as substrates. It is also important to note that the organism itself can be easily grown in natural conditions over the waste substrates.

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