

Production of Amylase and Xylanase Enzymes from Soil Fungi of Rajasthan

Mishra, BK*¹ and Dadhich, SK²

¹Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, MPUAT, Udaipur (Rajasthan)

²Krishi Vigyan Kendra, MPUAT, Sirohi (Rajasthan)

Abstract

The purpose of present investigation was to collect and isolate filamentous fungi from soil of different regions in Rajasthan state. Fifteen isolates were examined for their ability to produce amylase and xylanase. Among these, two isolates exhibited high enzymatic potential. *Aspergillus niger* RJ4 produced the highest extracellular amylase activity (196.4 U g⁻¹). *Trichoderma* sp RJ2 produced the highest levels of extracellular xylanase (140.8 U g⁻¹) activity. The xylanase produced by two of these isolates have good potential for pulp bleaching. This study contributes to catalogue soil fungi isolated in the state of Rajasthan.

Key words: amylase, xylanase, filamentous fungi

Introduction

Filamentous fungi are particularly interesting due to their easy cultivation, and high production of extracellular enzymes of large industrial potential. These enzymes have commercial application in various industries such as detergents, starch, drinks, food, textile, animal feed, baking, pulp and paper, leather, chemical and biomedical products. Use of starch degrading enzymes was the first large-scale application of microbial enzymes in the food industry¹. Amylases have applications in food, detergents, drinks, animal feed and baking². α -amylase carry out the conversion of starch to glucose, namely amylase, that cuts the large α -1,4-linked glucose polymers into shorter oligomers and glucoamylase hydrolyses the oligomers to glucose. Xylanases have potential application in food, feed, paper, pulp and textile industries³. These enzymes degrade plant fibers made of xylan hemicellulose producing xylose monomers. Xylanase is used in the pretreatment of pulps, prior to bleaching, in pulp and paper industries⁴. These enzymes release lignin fragments by hydrolyzing residual xylan and the pretreatment with xylanase reduces the usage of chlorine as the bleaching agent. It is also used for bread making and beer production. In this study we have

explored the amylase and xylanase production potential of several filamentous fungi, isolated from different soil samples. This study also contributes to cataloguing soil fungi isolated in the state of Rajasthan, in order to support future research about industrial application of these enzymes.

Experimental

Microorganisms and maintenance

The fungi used in this study were collected from samples of soil or decomposing organic material from several districts of the state of Rajasthan. The organisms were maintained on potato dextrose agar slants.

Culture conditions

The enzymes were obtained from cultures in liquid medium of composition adequate to the type of enzymatic activity of interest. For amylases we used Reese's mineral medium with 1% starch, and xylanase was obtained in Reese's mineral medium supplemented with 1% birchwood xylan. All flasks were inoculated with a spore suspension to give a final concentration of 4×10^5 spores mL⁻¹ of liquid medium, pH 6.0, at 30°C, under orbital agitation (100 rpm). After 5 days of incubation at 30°C the extracellular enzyme activities were determined in the culture filtrates as described.

Enzymatic assays and protein determination

Xylanase and amylase activities were assayed by measuring the reducing sugars released by dinitrosalicylic acid method⁵. For amylase, the reaction mixture consisted of 250 μ L of 1% (w/v) starch in sodium acetate buffer 100 mM, pH 5.0 and 250 μ L of enzyme. The reaction mixture was incubated at 60°C for 15 min and the reducing sugars formed were quantified by spectrophotometer at 540 nm. One unit was defined as the amount of enzyme that releases 1 μ mol of glucose per min under the assay conditions. Xylanase activity was determined using 1% birchwood xylan as substrate⁶. The reaction mixture was incubated at 50°C for 15 min. One unit was defined



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as the amount of enzyme that releases 1 mol of xylose per min under the assay conditions. Total soluble protein was also estimated in the crude extract using bovine serum albumin as standard⁷. All the enzymes were expressed as units per gram of soluble protein present in culture filtrates.

Statistical analysis

The data recorded in triplicates for the various observations were subjected to statistical analysis by the method of completely randomised block design using MSTAT-C statistical package and CD values were calculated at P = 0.058.

Results and Discussion

Fifteen strains of filamentous fungi were isolated from various regions of Rajasthan state, such as Udaipur, Dungarpur, Banswara, Rajsamand, Kota and Sirohi districts, from soil, in order to carry out a screen of potential biotechnological enzyme producers. Among fifteen fungi tested the *Aspergillus niger* RJ4 produced the highest extracellular amylase activity (196.4 U g⁻¹) followed by *Penicillium* sp RJ6 (145.8 U g⁻¹) whereas the lowest amylase activity was observed in case of *Fusarium* sp RJ2 (14.0 U g⁻¹) (Table 1). Decreasing trend of amylase activity in fungi as *A. niger* ITCC 2012 (392 U mL⁻¹) > *A. niger* NCIM 1054 > *A. oryzae* NCIM 533 (125 U mL⁻¹) was also reported⁹. The screening for xylanase activity was also performed in the same fifteen isolates. All fungi tested produced extracellular xylanases. *Trichoderma* sp RJ2 produced the highest levels of extracellular Xylanase (140.8 U g⁻¹) while *Aspergillus niger* RJ2 produced the lowest level of extracellular xylanase (9.5 U g⁻¹). *Fusarium* spp RJ1 and *Penicillium* sp RJ6, among others, were good producers of extracellular xylanases (Table 1). High xylanase activity is shown by two thermotolerant *Aspergillus* strains, identified as *A. caespitosus* and *A. phoenicis* who are thermostable at 50-55°C¹⁰. The optimum pH was 6.5 and 3.5 for *A. caespitosus* and *A. phoenicis*, respectively. The xylanases produced by *A. caespitosus* showed good performance for paper pulp bleaching.

Table 1 Extracellular amylases and xylanase activity (U g⁻¹) in different fungal isolates

S.No.	Fungal isolate	Amylase activity	Xylanase activity
1	<i>Aspergillus niger</i> RJ1	106.8	84.0
2	<i>Aspergillus niger</i> RJ2	25.0	9.5
3	<i>Aspergillus niger</i> RJ3	78.33	56.0
4	<i>Aspergillus niger</i> RJ4	196.4	78.0
5	<i>Aspergillus niger</i> RJ5	68.0	72.4
6	<i>Fusarium</i> sp RJ1	38.4	102.4
7	<i>Fusarium</i> sp RJ2	14.0	52.0
8	<i>Fusarium</i> sp RJ3	19.5	74.2
9	<i>Penicillium</i> sp RJ6	20.3	81.6
10	<i>Penicillium</i> sp RJ6	145.8	99.8
11	<i>Penicillium</i> sp RJ6	123.8	46.5
12	<i>Rhizopus</i> sp RJ1	56.2	36.5
13	<i>Rhizopus</i> sp RJ2	48.3	25.4
14	<i>Trichoderma</i> sp RJ1	31.5	94.5
15	<i>Trichoderma</i> sp RJ2	98.6	140.8
	SEm (±)	0.95	0.62
	CD at 0.05	3.8	2.45

Conclusions

In conclusion, fifteen isolates of filamentous fungi were obtained from soil and compost samples from different regions of Rajasthan state. Among these fungi, three exhibited enzymatic potential for industrial uses. Significantly higher levels of amylase and xylanase production were observed for the *Aspergillus niger* RJ4 and *Trichoderma* sp RJ2 respectively. Further studies are to be carried out with these isolated fungi for optimization of culture condition and characterization of the enzyme activity at various physico-chemical conditions so that potential of these new isolates for industrial uses may established.

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