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RESEARCH ARTICLE

Improved Xylanase Production by Mutated Fungal Strains Enhanced by Soc (Sesame Oil Cake)

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ABSTRACT: The influence of oil wastes for microbial xylanase production can use sesame oil cake (SOC) as substrate by the filamentous fungi *Aspergillus niger* and *Penicillium chrysogenum*. The wild and mutated strains of these organisms are grown in Mandel's medium with modified trace elements at ambient temperature with pH 6.0 for 6 days. Finally, the mutated strains of both the species produced xylanase in a higher amount. The maximum yield of Xylanase was from the UV mutated *Aspergillus niger* strain. The average production of Xylanase from this species is 0.0415IU/ml. Moreover, the Xylanase is found to be cellulase free on this substrate (SOC).
Keywords: SOC; *Aspergillus niger*; *pencillium chrysogenum*; wild & mutated strains, xylanase

INTRODUCTION

Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan [Maria Inês Rezende *et al*. (2002) into xylose, thus breaking down hemi cellulose, which is a major component of the cell wall of plants Okafor *et al*. (2007). In recent years many pulp and paper industry turned towards xylanase for bleaching of papers in inexpensive manner Kang *et al*. (1996, 1993).The use of xylanases in prebleaching of cellulose pulp has become an alternative approach in eliminating chlorine in bleaching and reducing chlorinated organic compounds in bleach plant effluents and it increase the brightness of pulp Eriksson, 1985;Viikari *et al*., 1994). Xylanase shows great potential for industrial application mainly in the bioconversion of lignocelluloses to sugar, ethanol and other useful substances, clarification of juices and wines, improving the nutritional quality of silage and green feed, treating animal feed to increase digestibility and the de-inking process of waste papers Bailey *et al*.(1993). In animal food industry, xylanases are used to encourage the body weight gains of animals (Silversides, 2000). In bread and bakery industry xylanases are used to increase the dough viscosity, bread volume and shelf life.

Microorganisms including bacteria, yeasts and filamentous fungi such as *Trichoderma, Bacillus, Cryptococcus, Aspergillus, Penicillium, Aureobasidium, Fusarium, Chaetomium, Phanerochaete, Rhizomucor, Humicola, Talaromyces* and many more produce xylanase Haltrich *et al*. (1996). In these, fungi produce xylanase enzymes extracellularly with a wide range of activities using various substrates both in submerged and solid state fermentation (SSF) processes (Mountfort, 1989). Extracellular enzymes ease the extraction procedure. SSF offers distinct advantages over submerged fermentation including economy of space needed for fermentation, simplicity of the fermentation media, no requirement for complex machinery, equipment and control systems, greater compactness of the fermentation vessel owing to a lower water volume, greater product yield, reduced energy demand, lower capital and low recurring expenditures in industrial operation, easier scale up processes, lesser volume of solvent needed for product recovery, superior yields, absence of foam build up and easier control of contaminants due to the low moisture level in the system (Suprabha, 2008).

The most economical way of Xylanase production is by solid state fermentation. Many solid substrates can be used for producing Xylanase (Jeya, 2005). SOC is one of the solid substrates which have high nutritional content for Xylanase production (Techapun, 2002). The particle size of oil cake was relatively smaller than the other substrates giving a higher surface area which would ease oxygen diffusion and nutrient absorption and assimilation by the mycelia. So, SOC is a good media for fungal growth (Pangpeikhang, 2005). The objective of our study was to use oil wastes for producing xylanase enzyme and to show improvement in production by using UV mutated *Aspergillus niger* strain**.**

2. EXPERIMENTAL SECTION

Sub culturing the stock culture:

Two strain Aspergillus *niger* and *Pencillium chrysogenum* were collected from, (SIMPRA), Thanjavur. The stock culture is sub cultured on PDA (potato dextrose agar) medium and incubated at room temperature for 2 days.

Solid state fermentation:

15g of substrate with 25ml of Mandel's medium is taken in a conical flask. The medium composition is that Distilled Water - 1000ml, Urea - 0.3g, Peptone - 0.75g, Yeast Extract - 0.25g $(NH_4)_2SO_4$ - 1.4g, KH_2PO_4- 2.0g, $CaCl_2$- 0.3g, $MgSO_4.7H_2O$ -0.3 g Trace elements(modified); $FeSO_4.7H_2O$ -5mg, $MnCl_2$-1.6mg.

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The medium and the trace elements were autoclaved separately. The flask was cooled down at room temperature and a known amount of sterilized trace elements was added. The flasks were then inoculated with Spores of the cultured fungi. Then the flasks containing the organisms were incubated for 6 days at the ambient temperature (28±3ºC).

Enzyme Extraction:

80ml of cold distilled water (4ºC) was added to the Solid State Fermentation medium (15 g substrate) after cultivation. The mixture was vigorously homogenized for 30 minutes at 200 rpm. The solid biomass residues were separated from the suspension by filtration through Whatmann filter paper No.1.The cell free supernatant was used as the source of crude enzyme preparation.

i) Estimation of protein and Xylanase:

The protein was estimated by Lowry's method. To 0.5ml of protein solution, 5.5ml of alkaline copper reagent was added and the contents are mixed well. It is allowed to stand at room temperature for 15 min. Then 0.5 ml of folin's reagent was added and mixed immediately. After 30 min, he colour developed was read at 640 nm, using spectrophotometer calibrated with reagent blank. The level of Xylanase produced can be assayed using this process. The enzyme solution was added to 2% xylan suspension in 100mM Tris HCl buffer (pH 7.0). Incubated at 55 ºC for 30min, then cooled with ice water. The insoluble xylan was removed by centrifugation and 0.5ml of supernatant was taken. Add 1ml of 3,5 dinitrosalicylate (0.5%) solution was added. The mixture was cooked in boiling water bath. Then the colour measured at 535nm.

ii) Strain improvement:

The two wild strains were used for mutational study. The stock fungal cultures were cultured into the newly prepared and sterilized Potato Dextrose Agar (PDA) medium. Then the fungal plates were kept under Ultra Violet (UV) radiation for about 2-3min. Then the mutated fungal plates are also incubated at room temperature for 2-3 days. After that the same Solid State Fermentation for enzyme production, enzyme assay methods and protein estimation methods are followed for mutational strains and the results are tabulated

3. Results and Discussion

3.1 Result

The figure shows the Xylanase activity of the culture supernatant of different strains such as wild and mutated *Aspergillus niger* and *Pencillium chrysogenum*. The Xylanase activities of the culture supernatants from the given media are mostly utilized by the mutated species. A very high Xylanase activity 0.0415IU/ml was seen in mutated *Aspergillus niger* this is about five times the maximum activity of other species. The results are shown in the below Table 1.

3.2 DISCUSSIONS

The result shows that UV mutated *Aspergillus niger* produces Xylanase enzyme when cultured in the media of Sesame Oil Cake (SOC). Most members of *Aspergillus niger* group are notable producers of extracellular enzymes including important plant cell wall hydrolyzing enzymes such as Xylanases (Gawande, 1999). *Pencillium chrysogenum* wild type has the least Xylanase production. The given optimum conditions did not suit this organism to produce higher level of Xylanase. The protein levels and Xylanase activities of the crude enzymes preparations from the carbon source however differed significantly. The level of protein secreted by wild strain of *Pencillium chrysogenum* in the media containing SOC was highest among the above said organisms. The lowest protein level was found in *Aspergillus niger* (mutated).

From the results, the highest Xylanase producing organism contain low protein level and the lowest Xylanase producing organism contain high level of protein. High cost of production of hydrolyzing enzyme is a limiting factor in the commercial and industrial applications. One area currently considered as cost reduction strategy is the use of waste materials as carbon sources for the production of enzymes. So, the waste materials from oil mills can be used as cheapest source for the ease production of Xylanase. In a recent research, carried out by Pandey *et al*. (1999) produced Xylanase by using *Aspergillus niger* USMAI 1 was found to be 9.5U/g substrate. In another study Xylanase was produced from *Pleurotus sp* using banana wastes (Reddy, 2003). Our data has shown that the mutated type strains of *Aspergillus niger* and *Pencillium chrysogenum* can produce extracellular proteins with significant Xylanase activity when cultivated in the media of Sesame Oil Cake (SOC).

4. CONCLUSIONS

The results obtained from this work strongly indicate that the SSF system using SOC as a substrate is an economical method for the production of Xylanase at extremely low operational cost based on the fact that SOC is one of the cheapest and abundant by-products of Sesame Oil industry. The cultivation system can easily be modified to enhance the productivity of the enzyme formation by the fungus which will facilitate the scale up processes for mass production. The Xylanase produced is characterized and is currently being used for the de-inking processes of Laser printed waste papers.

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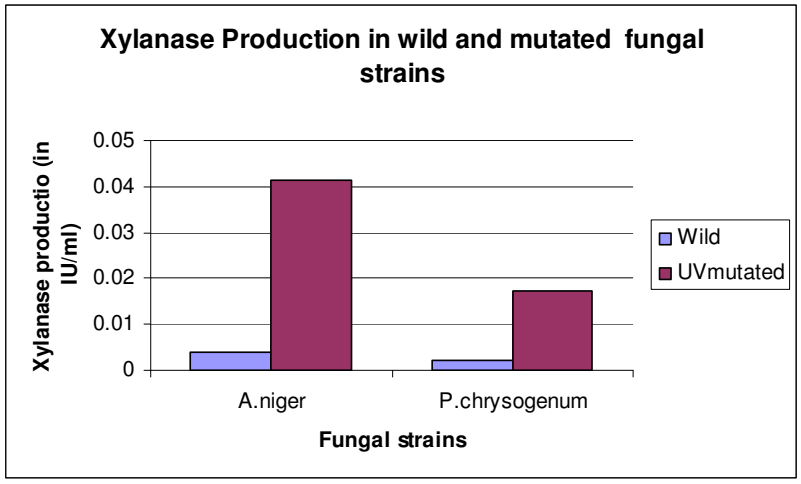
Table 1 Effect of wild and mutated *Aspergillus Niger* and *Pencillium Chrysogenum* on the production of xylanase enzyme.

Sl.No	Strain	*Aspergillus Niger* (IU/ML)	*Pencillium Chrysogenum* (IU/ML)
1	**Wild**	**0.0039**	**0.0021**
2	**UV-mutated**	**0.0415**	**0.0171**

Figure 1. Effect of wild and mutated *Aspergillus Niger* and *Pencillium Chrysogenum* on the production of xylanase enzyme.



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CONFLICTS OF INTEREST

"The authors declare no conflict of interest".