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ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.9, pp 4207-4211, September 2014

RTBCE 2014[12th August 2014]
Recent Trends in Biotechnology and Chemical Engineering

Xylanase Production from Aspergillus niger

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Abstract: Xylanase execute a large variety of function and have many important in biotechnological application. Its application in paper industry, pulp industry, increases the self life of bread, food ndustry. The production of xylanase was investigated in submerged culture of *Aspergillus niger*. This study was carried out in isolation and screening of xylanase enzyme from the *Aspergillus niger* fungus. This fungus is isolated from agricultural soil, decayed bread and waste material. The maximum concentration of xylanase enzyme found at 25^0c and also maximum production occurred when the pH was controlled at 6.0 during the fermentation. It have the maximum concentration. It has lot of industrial application.
Keywords: *Aspergillus niger*, xylanase, submerged culture.

Introduction

Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls.xylanases are present in fungi such as from the GRAS recognized fungus C1, Myceliophthora thermophila for the degradation of plant matter into usable nutrients. Commercial applications for xylanase include the chlorine-free bleaching of wood pulp prior to the papermaking process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting)[1-3].

Apart from its use in the pulp and paper industry, xylanases from commercially relevant fungi such as Myceliophthora thermophila, C1 and Trichoderma are also used as food additives to poultry, in wheat flour for improving dough handling and quality of baked products , for the extraction of coffee, plant oils, and starch, in the improvement of nutritional properties of agricultural silage and grain feed, and in combination with pectinase and cellulase for clarification of fruit juices and degumming of plant fiber sources such as flax, hemp, jute, and ramie.

Good number of scientific literature is available on key features of xylanase enzymes in biotechnology ranging from their screening in microbial sources to production methods, characterization, purification and applications in commercial sector are improper synthesis. A Variety of microorganisms, including bacteria, yeasts and filamentous fungi, have been reported to produce xylanases[3]. The potential application of xylanases with or without concomitant use of cellulase include the bioconversion liguno celluloses to sugar ethanol and

other useful substances, clarification of juice and wine, and nutritional value improvement of silage and green feed.

In addition, cellulase -free xylanases can be used for selective xydrolysis of the hemicelluloses components in paper and pulp. The use of purified xylan as an inducer increases the cost of enzyme production.For this reason ,different ligunocellulosic residues, including wheat bran, wheat straw ,corn cob and sugarcane bagasse, have been used as growth substrate in cultures to produce xylanases.

Large quantities of lingo cellulosic wastes are generated through forestry, agricultural practices and industrial processes, particularly from agro –allied industries such as breweries, pulp & paper, textile and timber industries. These wastes generally accumulate in the environment thereby causing pollution problem. Most of the wastes are disposed by burning, a practice considered as major factor in global warming. However the plant biomass regarded as "wastes" are biodegradable and can be converted in to valuable products such as biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients[5].

Lignocelluloses are mainly secondary plant cellwall materials which consists of lignin, cellulose and hemicelluloses . D-xylan is the major hemicellulose found in woods and accounts for 20-35% of the total dry weight of hardwood and perennial plants[15-17]. The basic structure of xylan is a β-D-(1,4)-linked xylopyranosyl residue with a few branch points. The major backbone carries relatively short side chains of variable lengths Due to the abundance and the structural heterogeneity of xylans, xylan degrading enzymes are diverse. Typical xylan – degrading enzymes are endo- β-xylanases (EC 3.2.1.8), which attack the main chain of xylans and β-xylosidases (EC 3.2.1.37), which hydrolyse xylooligo-saccharides in to D-xylose[14].

These two enzymes are required for complete hydrolysis of native cellulose and biomass conversion and are produced mainly by many bacteria and fungi. Potential application of xylanase in biotechnology include production of hydrolysates from agro industrial wastes, nutrient improvement of lignocellulosic feedstuff, clarification of juices and wines and biobleaching of kraft pulp in paper industry[9-11].

The present study was designed to investigate the potential use of some agro wates as carbon sources for xylanase production by the strains of *Aspergillus niger* and *Trichoderma viride* by SSF.In the present work the physio –chemical parameters of the media composition. Incubation time, temperature and pH were optimized for the production of total xylanase by SSF using the above two species utilizing agro industrial wastes namely wheat bran and sugarcane bagase[13].

Materials And Methods

Micro Organism Media And Culture Conditions

Aspergillus niger was isolated from soil. In the lab, the organism was maintained on potato dextrose agar slants at 30 degree Celsius. The spore suspensions were prepared by adding 10ml of sterilized water to slant cultures and the surface was gently rubbed with a sterilized wire loop. Enzyme production was studied in 250ml Erlenmeyer flasks containing 50ml of mineral solution and the appropriate carbon source. One mililiter of spore suspension was used as the inoculam. The cultures were incubated at 30 degree Celsius in a rotary shaker at 120rpm for 8 days . The cultures were harvested by filtration and the filtrates were saved as sources of crude extracellular enzymes.

Media And Culture Conditions

The salt medium for the growth of the fungal and the enzyme production was that of mandels and stenrnberg. The medium contained in each liter;

- KH2PO4(2.0g)
- (NH4)2SO4(1.4g)
- MgSO4-7H2O(0.3g)
- CaCl2(0.3g)
- Urea (0.3g)
- Tween-80(1ml)
- FeSO4.7H2O(5.0mg)
- MnSO4.H20(1.6mg)
- ZnSO4.7H20(1.4mg)

- CoCl2(2.0mg) and
- Corn-cob xylan (10g)

The pH was adjusted to 5.0. The addition of different substrates to the medium is indicated in each experiment. Cultivation mass made in 250ml Erlenmeyer flasks each containing 50ml of sterilized media.one milliliter of spore suspension obtained from 7day mother culture was used for inoculation. Cultivation was performed at 30 degree celcius on a rotary shaker (180rpm).the culture were harvested on the seventhday of growth by filtration through glasswool filter and then centrifuged. The clear supernatant were used for enzyme assays.

Determination Of Enzyme Activities

Xylanase activity in the culture filtrate was determined from the amount of the reducing sugars formed in terms of xylose according to the method of somogyi. The half milliliter of appropriately diluted culture filtrate was added to 0.5ml of 10% (w/v) xylan in 0.05M Phosphate buffer (pH 5.0). The reaction mixture was incubated at 45 degree celcius for 30 minutes. One unit(U) of xylanase activity was defined as the amount of enzyme liberating onemole of reducing sugars as a xylose per minute[8-9].

Results and Discussion

Screening Of Some Fungal Strains For The Production Of Extra Cellular Enzymes

All tested fungi were grown on the basal medium containing corn-cob xylan.The culture filterates were investigated for extracellular xylanase and β xylosidase activities on the 7th day of growth.*Aspergillus niger* was found to be the most potent fungus for xylanase and βxylosidase production, followed by *A.oryzae*. on the other hand ,*Fusarium oxysporum* 3A was comparatively the lowest xylanase producer.Therefore,*A.niger* was selected for further works.

Xylanase Production By A Wild Strain Of *Aspergillus Niger*

A. niger was grown in 250 mL Erlenmeyer flasks containing 50 mL mineral medium supplemented with different carbon sources (1%,w/v) for 5 days. Xylanase, CMCase and protease activities were determined in the early stationary growth phase., Low xylanase and no measurable amounts of CMCase were observed after growth on glucose, xylose, lactose, cellobiose and CMCellulose. High xylanase activities were detected when the microorganism was grown in commercial xylan (from birchwood and from oat spelt) and in lignocellulosic residues (corn cob, wheat bran, wheat straw, and sugar canebagasse).

A very significant improvement in the production of xylanase without concomitant increase in the CMCase and protease activities was observed when the substrate concentration in the media was increased from 1 to 3-5%. Low CMCase (∠ 0.05 U/mL) and protease (∠ 50 U/mL) activities were detected in these cultures, except in wheat bran cultures, where proteolytic activity up to 200 U/mL were found in the culture filtrates. For this reason, corn cob was the substrate selected to study the effect of cultivation time in the production of enzyme .

The maximum peak of xylanase production occurred at 6 days of cultivation, while very low CMCase and protease activities were detected, even after long time of cultivation. Xylanase activity was resistant to acetone precipitation. About 80% of initial activity was recovered after precipitation procedure. Some biochemical properties of crude xylanase were determined. Crude xylanase was most active between pH 5.0-6.0 and the enzyme was stable in pH range 3.5 to 10 after 4hrs of incubation at 40 degree Celsius.

The enzyme was stable at temperature up to 55^0 C, and it retained 50% and 23% of its initial activity when heated for 1h at 60^0 and 65^0 C, respectively The crude xylanase system was characterized by electrophoretic techniques. SDS-PAGE showed the presence of atleast four isoxylanases, with molecular weights of 50, 43, 20 and 18 kDa.

Optimization Of *A.Niger* Culture Conditions For Xylanase

Effect of pH of culture medium: The pH of culture medium was maintained at pH 3.0, 4.0, 5.0, 6.0and 7.0 through the fermentation process. Maximal enzyme production was detected at pH 6.0. These results coincide with those report for xylanase production by *A.tereus*, *xylanase* activity was 4.5 fold that at pH

3.0.The most significant effect of low Ph was demonstrated by the complete inhibition of β-xylosidase at pH 3.0.

The low enzyme activity at pH 3.0 may at least partially be due to inactivation of the enzyme at this pH rather than to inhibition of enzyme biosynthesis. This view may explain the production of appreciable amounts of extracellular protein at pH 3.0 and the large increase of enzyme yield the limited increase of extracellular protein and mycelia biomass at higher pH's. Therefore pH 6.0 was used.

The inductive nature of production of xylanase by *A. niger* was suggested by the high levels of enzyme found in cultures with xylan or lignocellulosic residues as substrate, whereas very low xylanase activities were detected on glucose, xylose, cellobiose and lactose cultures. The supplementation of cultures with 3-5% of lignocellulosic material improved the production of xylanase. The decreasing in the production of xylanase when high amounts of xylan was offered as carbon source, may be due to catabolite repression, likewise described for other xylanolytic microorganisms .

The strain of *A. niger* used in this work was able to produce levels of xylanase activity superior to those produced by other strain of *A. niger* In addition, the maintenance of xylanase activity after 8 days of cultivation, suggests that the enzyme produced by the fungus was stable and no proteolytic activity was co-produced by the fungus in the medium used in this work. The condition of temperature and pH for maximum xylanase activity was similar to other *Aspergillus* sp xyanases; However, the xylanases described here presented a rare stability in a large range of pH. Recently, a strain of *A. niger* able to produce a xylanase optimally active at pH 8.0 has been documented.

High activity and stability at alkaline pH is a very desirable property to use xylanases in selective hydrolysis of the hemicellulose components in paper and pulp. The occurrence of multiple xylanases in a microorganism immediately raises questions concerning the functions and origins of each isoenzyme.

Multiple xylanases production have been reported in numerous microorganisms and could be caused by several factors, such as different mRNA processing, partial proteolysis or differences in the degree of amidation and glycosilation. Multiple forms of xylanases differ in stability, catalytic efficiency, and absorption and activity on substrates .

Different types of xylanases might allow the microorganisms to use a wider range of substrates or allow their diffusion into the plant cell walls of highly variable structure In this work, we described some basic information for the production of high titles of xylanase by a wild strain of *A. niger* isolated from soil. Its xylanase activity is cellulase-free and can be produced on cultures using a very cheap growthsubstrate.

References

1. Archana, A. and Satyanarayana, T. Xylanase production by thermophilic Bacillus licheniformis A99 in solid-state fermentation, Enzyme and Microbial Technology., 1997, 21, 12–17.
2. Bailey, M. J.; Biely, P. and Poutanen, K. Interlaboratory testing ofmethods for assay of xylanase activity, Journal of Biotechnology., 1992, 23, 257–270.
3. Bailey, M. J. and Poutanen, K. Production of xylanolytic enzymes by strains of *Aspergillus*, Applied Microbiology and Biotechnology., 1989, 30, 5–10.
4. Bailey, M. J.; Puls, J. and Poutanen, K. Purification and properties of two xylanases from *Aspergillus oryzae*, Biotechnology and Applied Biochemistry., 1991, 40, 224–229.
5. Bandivadekar, K. R. and Deshpande, V. V. Enhanced stability of cellulase-free xylanase from *Chainia sp*, Biotechnology Letters., 1994, 16, 179–182.
6. Biswas, S. R.; Jana, S.C.; Mishra, A. K. and Nanda, G. Production, purification and characterization of xylanase from a hyperxylanolytic mutant of *Aspergillus ochraceus*, Biotechnology and Bioengineering., 1990, 35, 244–251.
7. Biswas, S. R.; Mishra, A. K. and Nanda, G. Xylanase and b-xylosidase production by *Aspergillus ochraceus* during growth on lignocelluloses, Biotechnology and Bioengineering., 1988, 31, 613–616.
8. Cai, J. M.; Wu, K.; Yu, J.; Zhang, J.; Tang, B. and Pan, R. R. Production of xylanase during solid state fermentaion of *Penicillium sp*. strain P1, Food and Fermentation Industries, 1997, 23, 30–33.
9. Cesar, T. and Mrsa, V. Purification and properties of the xylanase produced by Thermomyces lanuginosus, Enzyme and Microbial Technology., 1996, 4, 289–296.

10. Christov,L. P.; Szakacs, G. and Balakrishnan, H. Production, partial characterization and use of fungal cellulase-free xylanases in pulp bleaching, Process Biochemistry., 1999, 34, 511–517.
11. Ferreira, G.; Boer, C. G. and Peralta, R. M. Production of xylanolytic enzymes by *Aspergillus tamarii* in solid state fermentation, FEMS Microbiology Letters., 1999, 173, 335–339.
12. Gawande, P. and Kamat, M. Y. Preparation, charaterization and application of *Aspergillus sp.* xylanase immobilized on Eudragit S-100, Journal of Biotechnology., 1998, 66, 165–175.
13. Gawande, P. and Kamat, M. Y. Production of Aspergillus xylanase by lignocellulosic waste fermentaion and its application, Journal of Applied Microbiology, 1999, 87, 511–519.
14. Gerber, P. J.; Heitmann, J. A. and Joyee, T. W. Purification and characterization of xylanases from Trichoderma, Bioresource Technology., 1997, 61, 127–140.
15. Ghose, T. Measurement of cellulase activities, In Commission on Biotechnology, 1994,1–12.
16. Grajek, W. and Gervais, P. Influence of water activity on the enzyme biosynthesis and enzyme activities produced by Trichoderma viride TS in solid state fermentation, Enzyme and Microbial Technology, 1987, 658–662.
17. Haapala, R.; Parkkinen, E.; Suominen, P. and Linko, S. Production of endo-1,4-b-glucanase and xylanase with nylon web immobilized and free Trichoderma reesei, Enzyme and Microbial Technology, 1996, 18, 495–501.

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