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(RESEARCH ARTICLE)

Effect of optimization conditions on submerged fermentation of corn bran for the production of xylanase enzyme

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Abstract

UV- rays were used as mutagens on wild type of *Aspergillus niger* for enhanced production of Enzyme – Xylanase. The xylanase producing species were isolated from soil and exposed to UV rays at various timings of 20 mins, 40 mins, 60 mins, 80 mins, 100 mins and 120 mins. The mutants AN20 – AN100 showed 19.96, 21.22, 22.66, 27.88, and 37.77 % increase in activities of cellular xylanase respectively in comparison with the parental strain (0.556± 0.06). Mutant AN100 exposed to UV ray for 100 minutes was therefore selected as the best producer of xylanase after 7 days of fermentation using Corn bran as a substrate. As seen in the result, increase in time of exposure resulted to a decrease in cellular xylanase production. Optimization of xylanase production was carried out and the result obtained revealed that mutant AN100 showed best xylanase activity when cultivated at P^H 5, 30 °C and without addition of any other Carbon or Nitrogen source. *Aspergillus niger* is a good producer of xylanase under the conditions determined in this assay

Keywords: Submerged fermentation; Xylanase; Optimization; *Aspergillus niger*

1. Introduction

Xylanases are enzymes that degrade xylan, a hemicellulose found in plant cell walls into xylose. Hemicellulose is the second abundant renewable biomass in nature. Xylan is the major hemicellulose component and approximately accounts for 20-25% of plant cell wall dry weights [1]. They are very important class of enzymes used in paper and pulp industry. The basic structure of xylan is ß-D-1, 4-linked xylopyranosyl residue with a few relatively short side chains. The heterogeneity of xylan led to a diversity of xylan-degrading enzymes.

Hitherto, the removal of lignin from paper and pulp was by chlorine and its compounds which have caused serious problem to health and environment. However, delignification by xylanase is an alternative approach, which is environmentally friendly [2].

Xylanases are of considerable interest because of their applications in bleaching of pulp in the paper industry, food processing, bio-conversion of biomass wastes to fermentable sugars and clarification of fruit juices [2]. They also have application in improvement, nutrient digestibility in animal diets and in the production of 3-xylooligosaccharides and xylose.

Apart from its use in the pulp and paper industry, xylanases 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.

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Commercial applications for xylanase include the chlorine-free bleaching of wood pulp prior to the paper making process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting)

Large quantities of hemicellulosic wastes can be generated through forestry, agricultural practices and industrial processes, particularly from agro –allied industries such as breweries, pulp and paper, textile and timber industries. These wastes generally accumulate in the environment thereby causing pollution and other environmental hazards. 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 into valuable products such as biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds, human nutrients and production of enzymes such as xylanases.

A Variety of microorganisms, including bacteria, yeasts and filamentous fungi, have been reported to produce xylanases and over the years, a number of organisms of the genus Penicilium, Trichoderma, Pleurotus, Aspergillus, Streptomyces and Bacillus have been manipulated for xylanase biosynthesis [3,4]. However, *Aspergillus niger* has been described as the most potent organism for xylanase production [2]

In this study effort was made to improve xylanase production from mutagenization of *A. niger* as a means of obtaining isolates with high yields of xylanase. The mutants were compared with the parental strain for the production of xylanase using corn bran as a substrate.

2. Material and methods

2.1. Collection of samples

Corn bran was collected from a local milling house located in Gonin-gora market in Kaduna metropolis, while Soil sample was collected from one inch below the soil surface in sterile bag from the waste dump site behind Federal Government College – Kaduna state in Nigeria. Samples were transferred into a sterile polyethylene bag and dispatched to the Microbiology Laboratory, Kaduna State University, Nigeria and kept at ambient temperature till use.

2.2. Isolation of fungi for xylanase production

One milliliter (1 ml) of the serially diluted soil sample was poured into sterile petri dishes containing Potato Dextrose Agar (PDA) medium. Streptomycin (0.05 g) was added to the molten PDA medium in the petri dishes and incubated at 30±1 °C for 5 days for the isolation of Aspergillus spp. Preliminary identification of the fungi was done based on their morphological characteristics. The dominant isolates of *Aspergillus niger* was sub-cultured in PDA medium. The isolated Aspergillus spp was purified by restreaking on the medium and the pure cultures were maintained on PDA slants stored at 4 °C in the refrigerator.

The isolated fungal culture was identified as *Aspergillus niger* based on colonial morphological and microscopic characteristics and these values matched with values in standard reference book compendium of soil fungi [5].

2.3. Screening of *A. niger* isolates for xylanase production

Screening for the production of xylanase was performed on Potato Dextrose agar (PDA) containing 0.1% (w/v) of commercial xylan. Positive xylanolytic isolates were detected based on the clear zones of hydrolysis of the xylan [6]. The potential isolates were sub cultured and maintained on PDA slants. The slants were then stored at 40c prior to use.

2.4. Modification of *A. niger* isolates by UV treatment for enhanced xylanase production

Various serial dilutions of fungal suspension were prepared and dilution10^{-2}, 10^{-3} and 10^{-4} were distributed into sterilized petri plates (2 ml in each plate). These were exposed to UV radiations for varying time periods ranging from 20 to 120 minutes in UV chamber keeping the distance of UV source at 15 cm. After UV radiation they were kept in dark for stabilization of thymine thymine (T-T) dimmers. Parent type and UV treated fungal spore suspensions of 0.1 ml was inoculated into 25 ml petri plate containing potato dextrose agar medium. Then developed fungal strains whose survival rate was less than 1% were screened on PDA containing 0.1% (w/v) of commercial xylan for hyper- xylanase activity.

2.5. Xylanase production using selected mutants and parent *A. niger* on corn bran

2.5.1. Inoculum preparation for xylanase production

Spores were harvested by flooding the plates with sterile distilled water containing 0.05% Tween 80 as a wetting agent, after which spores were scraped from the surface of the colonies with a sterile spatula. The resulting suspension was shaken in a 100 ml Erlenmeyer flask to break up the spore chains. The concentrations of spores was determined using a hemocytometer, after which the suspension was further diluted in sterile Tween 80 solution to achieve the desired concentration (10^7spore per ml).

2.5.2. Substrate preparation and culture condition

The corn bran was pounded into coarse particle sizes, washed exhaustively with warm water, then air dried to eliminate moisture and make it susceptible to ball milling [7]. Milled sample was then sieved to particle size of 850u to be used for all experimentation. The basal medium for enzyme production was prepared by introducing corn bran (10g) into 100ml Erlenmeyer flask with the nutritive solution containing in each liter; KH_2PO_4(2.0 g) ,$(NH_4)_2SO_4$(1.4 g), $MgSO_4$-$7H_2O$(0.3 g), $CaCl_2$(0.3 g), Urea (0.3 g), Tween-80(1 ml), $FeSO_4.7H_2O$(5.0 mg), $MnSO_4.H_20$(1.6 mg), $ZnSO_4.7H_20$(1.4 mg), $CoCl_2$(2.0 mg).

The pH was adjusted to 5.0. [8]. Cultivation mass made in 100ml Erlenmeyer flasks, each containing 50ml of sterilized media. One milliliter of spore suspension obtained from 7day mutant cultures was used for inoculation. Cultivation was performed at 30 degree Celsius on a rotary shaker (180rpm). The culture was harvested on the seventh day of growth by filtration through Whiteman filter paper and then centrifuged. The clear supernatant was used for enzyme assay.

2.6. Enzyme assay of xylanase

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 [8]. Half milliliter of the culture filtrate was added to 0.5 ml of 10% (w/v) xylan in 0.05 M Phosphate buffer (pH 5.0). The reaction mixture was incubated at 45 ^{0}C for 30 minutes. The release of the reducing sugars was determined by the dinitrosalicylic acid method according to [9]. One unit (U) of xylanase activity was defined as the amount of enzyme liberating one mole of reducing sugars as xylose per minute. The activity of Xylanase enzyme was determined as IU/ml. Enzyme activity was calculated from the amount of reducing sugar produced in 30 minutes, using the formula.

$$\text{Enzyme activity IU/ML} = \frac{\text{Amount of reducing sugar} \times 1000 \times 1}{\text{Molecular weight of glucose} \times \text{time} \times \text{enzyme volume}}$$

2.7. Optimization studies for enzyme production

Various physiochemical factors affecting enzyme production such as source of carbon or sugar fermentation, source of Nitrogen, Temperature condition and pH condition were optimized for maximum yield [8].

2.7.1. Effect of additional carbon sources

Different carbon sources were tested for their ability to enhance xylanase production, they are (Xylose, Fructose, Maltose, Mannitol, Sucrose, Galactose, Lactose and Glucose) which were added to the fermentation medium in ratio 1 % (w/v) separately and incubated for 7days at 30^{0}C

2.7.2. Effect of additional nitrogen sources

Nitrogen sources (organic and inorganic) were also tested for the ability to enhance Xylanase production. These include Yeast extract, Sodium nitrate (Na_2NO_3), Ammonium Sulphate (NH_4SO_4), and Urea which were added to the fermentation medium in ratio 0.5% (w/v) separately and incubated for 7 days at 30 ^{0}C

2.7.3. Effect of fermentation temperature

In order to determine the optimum temperature for Xylanase production, the culture medium was properly inoculated and incubated for 7 days at various temperature 20, 25, 30, 35, and 40 ^{0}C.

2.7.4. Effect of fermentation pH

In order to determine the optimum pH for the production of xylanase, the fermentation broth was adjusted to various pH such as 4, 4.5, 5.5, 6 and 6.5. After 7 days fermentation, enzyme activity was determined and compared with the control.

3. Results and discussion

3.1. Spectroscopic determination of xylanase activity of *A. niger*

Table 1 Effect of time of UV-induce mutant strain of *A. niger* on xylanase production from SmF of corn bran

Code of organisms	Time of exposure (minutes)	Enzyme activities (iu/ml)
WT	0	0.556± 0.06 b
AN20	20	0.667± 0.04 c
AN40	40	0.674± 0.04 c
AN60	60	0.682± 0.03 c
AN80	80	0.711± 0.06 d
AN100	100	0.766± 0.01 d
AN120	120	0.444± 0.04 a

Code: WT= parent strain of *Aspergillus niger*, AN20 – AN100 = Mutant strains of *Aspergillus niger*. Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

Modification of *Aspergillus niger* by UV treatment had a positive effect on the production of xylanase. The duration of exposure was directly proportional to the enzyme activity of the mutants. The enzyme production from the mutant strain increased significantly with increased time of UV exposure from 20 minutes to 100 minutes after which the production declined significantly at 120 minutes of UV exposure. This result revealed that mutant exposed to 120minutes exhibited the least enzyme activity. Exactly 37.77% increase in enzyme activity was recorded when mutant AN100 (exposed to 100minutes) was used, in comparison with the parent strain. This result is in agreement with that of [10] who used UV treatment on *Aspergillus niger* to obtain an increase of 82.31% in glucose oxidase production. However, [11] and [12] reported maximum enzyme activity after 45minutes and 25minutes of UV irradiation respectively.

3.2. Optimization condition for sugar fermentation

Table 2 Effect of different carbon sources on xylanase production from SmF of corn bran

Carbon sources	Enzyme activity (iu/ml)
Xylose	0.088 ± 0.01f
Fructose	0.040 ± 0.00d
Maltose	0.007 ± 0.00a
Mannitol	0.074 ± 0.01e
Sucrose	0.030 ± 0.00c
Galactose	0.070 ± 0.01e
Lactose	0.015 ± 0.01b
Glucose	0.082 ± 0.02f

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

The mutant strain of *Aspergillus niger* (AN100), used in this study showed different enzyme activity with additional sugars to the fermentation broth. The sugar consists of Xylose, Fructose, Maltose, Mannitol, Sucrose, Galactose, Lactose and Glucose (Table 2). The result shows that Xylose exhibited the best enzyme activity of 0.088 iu/ml while Maltose had the least enzyme activity of 0.007 iu/ml. However, an inhibition of enzyme activity was observed with additional

carbon sources. This result disagrees with the findings of [6] who reported maximum enzyme activity (25.40u/g) with supplementation of Xylose in solid state fermentation of Palm kernel cake using *Aspergillus niger*. The result however corroborates the results obtained by [13] who attributed such inhibitory effect to catabolic repression process. The findings of [8] also showed inhibitory actions of fermentative sugars on xylanase production while detecting an increase in enzyme activity with the supplementation of culture with 3-5% of lignocellulosic materials.

3.3. Enzyme assay for nitrogen optimization

Table 3 Effect of different Nitrogen sources on xylanase Production from SmF of Corn Bran

Nitrogen sources	Enzyme activity (iu/ml)
Yeast extract	0.63 ± 0.02 b
Na2NO3	0.64 ± 0.01 b
NH4SO4	0.59 ± 0.04 a
Urea	0.65 ± 0.01 b
Control	0.766± 0.01

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

The highest enzyme activity was observed in urea with 0.65 iu/ml, followed by Na_2NO_3 with 0.64 iu/ml, yeast extracts with 0.63 iu/ml and NH_4SO_4 with 0.59 iu/ml respectively (Table 3). This showed that organic source of nitrogen was the best source of nitrogen for xylanase production. Effectiveness of inorganic nitrogen compounds on Xylanase production has also been reported by [6]. However, the result in table 1 indicates that Corn bran alone is more suitable for xylanase production. The mutant *A. niger* (AN100) used in this study reaches the maximum xylanase activity (0.766 iu/ml) in the absence of additive nitrogen to the main medium. The richness of natural Corn bran with protein, Carbohydrate and Fiber as reported by [17] may be enough to stimulate growth and enhance xylanase production. This result disagrees with the finding of [14] who reported that *A. niger* requires Na_2NO_3 to enhance xylanase production. Some other fungi such as *T. viride* as reported by [2] require ammonium (HN_4) residue to improve xylanase production.

3.4. Optimization condition for temperature

Table 4 Effect of temperature on xylanase production from SmF of corn bran using mutant strain of *A. niger.*

Temperature (0C)	Enzyme Activities (iu/ml)	
20	0.059±0.01b	
25	0.089±0.01c	
30	0.766±0.01e	control
35	0.415±0.09d	
40	0.037±0.01a	

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

The mutant strain of *Aspergillus niger* (AN100) used in this study showed that the optimum incubation temperature for production of xylanase enzyme was 30^0C with enzyme activity of 0.766iu/ml. Xylanase production was extremely low at 40^0C (Table 4). The result obtained is similar to those obtained by other authors who established that the best temperature range for xylanase activity is between 20^0C to 30^0C [13 15 and 16]. At a lower temperature of 20^0C, a lower enzyme activity of 0.059 iu/ml was recorded. Lower temperature decreases enzyme production because it is unsuitable for mold growth and as a result, lowers enzyme production while higher temperature limits oxygen concentration and leads to reduction in media water content by vaporization thereby affecting cell growth and consequently decreases enzyme activity. For *A. niger* (AN100), the optimum temperature for xylanase production was similar to the temperature of natural habitat of the parent strain where it was initially isolated. This corroborates the result of [6] who showed that the highest xylanase activity was obtained at temperature that was optimum for growth of the fungus in solid state fermentation.

3.5. Enzyme assay for pH optimization

Table 5 Effect of pH on xylanase Production from SmF of Corn Bran using mutant strain of *A. niger*

pH	Enzyme activities (iu/ml)
4	0.563± 0.10 b
4.5	0.526± 0.16 b
5	0.776± 0.01 b
5.5	0.578± 0.09 b
6	0.563± 0.08 b
6.5	0.444± 0.09 b

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

The highest enzyme activity in this study (0.766 iu/ml) was obtained at pH 5 while the least activity of 0.444 iu/ml was observed at pH 6.5, using the mutant strain of *A. niger* (AN100). This result disagrees with the findings of [14] who reported that the best xylanase production (1.48 u/ml) was at pH 6 when *A. niger* was used in solid state fermentation of wheat straw. Fluctuation in enzyme production was observed as the pH increases (Table 5). The low enzyme activity at pH 4 may partially be due to inactivation of the enzyme at this pH rather than inhibition of enzyme biosynthesis.

4. Conclusion

From this research work *Aspergillus niger* was isolated from soil. Modification of this wild type of *A. niger*, using UV-rays at 100 minutes resulted to a mutant with a higher ability to produced xylanase enzyme in a submerged fermentation of Corn bran. Optimization of xylanase production indicated that the mutant showed best xylanase activity when cultivated at P^H 5, 30 °C and without addition of any other carbon or nitrogen source. Mutation of *Aspergillus niger,* using UV treatment enhances production of xylanase enzyme under the conditions determined in this assay.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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