Research Paper

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Optimization of Culture Conditions for Production of Xylanase from *Thermomyces Lanuginosus* by Liquid State Fermentation

Botany

KEYWORDS : Xylanase, Culture conditions, Wheat bran, Yeast extract, Detergents, Additives.

Seema Rawat

Department of Botany and Microbiology H.N.B. Garhwal (Central) University, Srinagar, Pauri Garhwal, Uttarakhand, India

ABSTRACT

Xylanase is an important enzyme having various biotechnological applications. The present work focused on optimization of culture conditions for enhanced enzyme production. The maximum production was observed at 400C (15.20□0.20 U/ml) after 96h of incubation (15.20 0.20 U/ml) and at pH 6.5 (15.20 0.20 U/ml. Maximum enzyme production was observed on wheat bran as substrate (15.20 0.20 U/ml) and at 1.5% wheat bran (18.10 0.10 U/ml. Maximum xylanase production was observed on yeast extract (18.10 0.10 U/ml) and at 1.5% level (20.10 0.10 U/ml). All additives and detergents decrease the activity of enzyme. Maximum decrease was observed when tween-80 was added (10.15 0.21U/ml). Ca2+ and Mn2+ were found to enhance xylanase production.

1. Introduction

Xylanases of thermophilic fungi always draw attention due to their wide applications viz., processing of paper and pulp, animal-feed, wine, textile, pharmaceuticals and solid waste management (Kuhad *et al*., 1997; Rawat and Johri, 2002; Rawat and Johri, 2004; Gaffney *et al*., 2009). Xylanases from various thermophilic fungi have been well studied by many workers (de Almedie *et al*., 1995, Dusterhoft *et al*., 1997, Prabhu and Maheshwari, 1999, Gaffney *et al*., 2009). Strain variability in enzyme productions has also been reported (Cesar and Mrsa, 1996; Chadha *et al*., 1999). Xylanase biosynthesis is known to be regulated by induction and catabolite expression. Most xylanases are active between pH 4.5 to 6.5, at temperatures between 55^{0}C to 65^{0}C (Prabhu and Maheshwari, 1999). There are also reports of occurrence of multiple forms of xylanases in thermophilic fungi differing in their molecular size and kinetics (Coughlan and Hazlewood, 1993; Dusterhoft *et al*., 1997). The present research work was aimed at characterization of xylanases from *Thermomyces lanuginosus*, a thermophilic fungus.

2. Materials and Methods

2.1 Inoculum

Thermomyces lanuginosus was cultured on Yeast phosphate soluble starch medium for 4days at 40±1^{0}C. Two agar discs (4.0mm diamter, 2.0 mm thick) were cut and used as inoculum for the flasks.

2.2 Liquid –state fermentation (LSF)

Isolate was grown in triplicate for 4d in 50ml xylanase production medium (XPM) containing 1% wheat bran, 0.1% yeast extract; 0.2% peptone, 0.1% K_2HPO_4 and 0.05% $MgSO_4.7H_2O$, pH 6.5 in 250 ml flasks in an incubator shaker at 40±1^{0}C, 180 rpm.

2.3 Extraction of crude enzyme

The contents from flasks were centrifuged at 10,000rpm for 10min, 4^{0}C and supernatant was used as crude enzyme.

2.4 Enzyme assay

Xylanase activity was determined using birchwood xylan as substrate. The reaction mixture, containing 0.5 ml of 1% birchwood xylan in sodium phosphate buffer (0.1 M, pH 6.0) and 0.5 ml of crude enzyme, was incubated at 50^{0}C for 60 min in water bath. The liberated sugars were estimated according to Miller (1959). The activity of enzyme was expressed as the amount of enzyme releasing 1 µ mole of xylose per ml per min.

2.5 Optimization of culture conditions for xylanase production

Effect of temperature on xylanase production

The fungus was grown in XPM at different temperatures, ranging from 30^{0}C to 60 ^{0}C and xylanase production was monitored.

Effect of incubation period on xylanase production

The fungus was grown in XPM at 40^{0}C and xylanase production was monitored at different time periods ranging from 0 to 120 h by withdrawing aliquots.

Effect of pH on xylanase production

Xylanase production was monitored at different pH of XPM ranging from 3.0 to 10.0.

Selection of best carbon source for enzyme production

Various agro-residues viz., wheat bran, wheat straw, rice straw and sugarcane bagasse at 1% (w/v) level, were used to select the best carbon source for maximum xylanase production by the fungus.

Effect of different levels of wheat bran on xylanase production

Varying amounts of wheat bran were incorporated in XPM and enzyme production was monitored.

Selection of best nitrogen source for xylanase production

Various organic and inorganic nitrogen sources were screened at 1% (w/v) to select the best source that supports maximum xylanase production.

Effect of different levels of yeast extract on xylanase production

Varying amounts of yeast extract were incorporated in XPM to monitor enhanced xylanase production.

Effect of additives and detergents on xylanase production

Additives (EDTA, 0.01% w/v) and detergents (Tween-80, 0.1% w/v; triton-X100, 0.02% w/v; SDS, 0.001% w/v) were used as supplements to xylanase production medium to evaluate their effect on enzyme production.

Effect of divalent cations on xylanase production

The divalent cations such as Mg^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+} were added to XPM as $MgSO_4$, $FeSO_4$, $MnSO_4$ and $CuSO_4$ (0.01% w/v) to study their effect on enzyme production.

3. Results

3.1 Effect of temperature on xylanase production

The maximum production was observed at 40^{0}C (15.20±0.20 U/ml) and minimum at 30^{0}C (2.10±0.18 U/ml).

Table 1: Effect of temperature on xylanase production by *Thermomyces lanuginosus*

Temperature (^{0}C)	Activity (U/ml)
30	2.10±0.18
35	8.20±0.14
40	15.20±0.20
45	10.14±0.10
50	6.45±0.10
55	4.17±0.10
60	15.74±0.35

Incubation period- 96 h; pH- 6.5

3.2 Effect of incubation period on xylanase production

Maximum xylanase production was observed after 96h of incubation (15.20±0.20 U/ml) and no production after 12h of incubation

Table 2: Effect of incubation period on xylanase production by *Thermomyces lanuginosus*

Incubation period(h)	Activity (U/ml)
12	-
24	0.45±0.10
36	2.09±0.11
48	4.56±0.16
60	7.24±0.10
72	10.60±0.16
84	12.40±0.10
96	15.20±0.20
108	10.40±0.12
120	5.21±0.14

Incubation pH- 6.5; temp.- 40^{0}C

3.3 Effect of pH on xylanase production by *Thermomyces lanuginosus*

Maximum xylanase production was observed at pH 6.5 (15.20±0.20 U/ml) and no production was observed at pH 3.0, 3.5 and 10.0

Table 3: Effect of pH on xylanase production by *Thermomyces lanuginosus*

pH	Activity (U/ml)
3.0	-
3.5	-
4.0	0.26±0.07
4.5	1.10±0.10
5.0	5.21±0.10
5.5	8.21±0.20
6.0	10.63±0.12
6.5	15.20±0.20
7.0	12.18±0.11
7.5	10.28±0.14
8.0	08.29±0.19
8.5	3.21±±0.10
9.0	0.37±0.10
10.0	-

Incubation period- 96 h; temp.- 40^{0}C

3.4 Selection of best carbon source for enzyme production

Maximum enzyme production was observed on wheat bran as substrate (15.20±0.20 U/ml) and least production on rice straw (7.56±0.20 U/ml).

Table4: Effect of carbon source on xylanase production by *Thermomyces lanuginosus*

Carbon Source	Activity (U/ml)
Wheat straw	9.56±0.26
Wheat bran	15.20±0.20
Sugarcane bagasse	12.67±0.10
Rice straw	7.56±0.20

Incubation temperature : 45^{0}C, period 96 h; pH 6.5

3.5 Effect of different levels of wheat bran on xylanase production

Maximum xylanase production was observed at 1.5% wheat bran (18.10±0.10 U/ml) and least at 3.0% (5.12±0.10 U/ml).

Table 5: Effect of different levels of wheat bran on xylanase production by *Thermomyces lanuginosus*

Level (%)	Activity (U/ml)
0.5	9.12±0.12
1.0	15.20±0.20
1.5	18.10±0.10
2.0	10.35±0.20
2.5	7.23±0.10
3.0	5.12±0.10

Incubation temp.- 45^{0}C; period- 96 h; pH- 6.5

3.6 Selection of best nitrogen source for xylanase production

Maximum xylanase production was observed on yeast extract (18.10±0.10 U/ml) and least on potassium nitrate (0.24±0.12 U/ml).

Table 6: Effect of nitrogen source on xylanase production by *Thermomyces lanuginosus*

Nitrogen source (%)	Activity (U/ml)
Yeast extract	18.10±0.10
L-asparagine	15.21±0.11
Peptone	14.23±0.13
Tryptone	12.12±0.22
Beef extract	14.24±0.09
Casein	2.46±0.26
Malt extract	6.42±0.15
$(NH_4)_2HPO_4$	13.55±0.20
$(NH_4)_2SO_4$	13.55±0.20
KNO_3	0.24±0.12

Incubation temp.- 45^{0}C; period- 96 h; pH- 6.5; wheat bran (1.5%)

3.7 Effect of different levels of yeast extract on xylanase production

Maximum xylanase production was observed on 1.5% yeast extract (20.10±0.10 U/ml) and least on 3% (8.37±0.21 U/ml).

Table 7: Effect of different levels of yeast extract on xylanase production by *Thermomyces lanuginosus*

Level (%)	Activity (U/ml)
0.5	12.69±0.12
1.0	15.0±0.20
1.5	20.10±0.10
2.0	15.98±0.23
2.5	10.43±0.10
3.0	8.37±0.21

Incubation temp.- 45^{0}C; period-96 h; pH- 6.5

3.8 Effect of additives and detergents on xylanase production

All additives and detergents decrease the activity of enzyme. Maximum decrease was observed when tween-80 was added (10.15±0.21U/ml).

Table 8: Effect of additives and detergents on xylanase production by *Thermomyces lanuginosus*

Supplement	Activity (U/ml)
Control	20.10±0.10
Tween-80 (0.1%)	10.15±0.21
Triton X-100 (0.02%)	11.20±0.30
SDS (0.001%)	12.10±0.20
EDTA (0.01%)	15.25±0.20

Incubation temp.- 45^{0}C; period-96 h; pH- 6.5

3.9 Effect of divalent cations on xylanase production

Ca^{2+} and Mn^{2+} were found to enhance xylanase production while Mg^{2+}, Fe^{2+} and Cu^{2+} were found to decrease xylanase production. Maximum production was observed when Ca^{2+} was supplement-

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ed into the medium (25.04±0.45 U/ml) and least when Cu^{2+} was added (14.05±0.20 U/ml).

Table9: Effect of divalent cations on xylanase production by *Thermomyces lanuginosus*

Supplement (0.01%)	Activity (U/ml)
Control	20.10±0.10
Ca^{2+}	25.04±0.45
Mn^{2+}	23.23±0.20
Mg^{2+}	15.32± 0.12
Fe^{2+}	16.10± 0.15
Cu^{2+}	14.05±0.20

Incubation temp.- 45^{0}C; period-96 h; pH- 6.5

4. Discussion

Xylanase production was substantially high on wheat bran. Wheat bran has been reported to support xylanase production in many thermophilic moulds like *Humicola lanuginose* and *Thermoascus auranticus* (Kitprerchavanich, 1984; Grajek, 1987; Alams *et al*., 1994; Gaffney *et al*., 2009; Su *et al*., 2011). However, the level of enzyme in LSF was higher than those reported for *H. lanuginosa* (13U ml^{-1}) (Anand *et al*., 1990). Wheat bran and sugarcane baggase both possess high hemicellulose content. Wheat bran has been found to support xylanase production without other carbon and/or nitrogen supplementation, but there are instances where this substrate was not effective, viz., *Sporotrichum* sp. and *Myceliophthora thermophilum* (Dubey and Johri, 1987; Gaffney *et al*., 2009; Simoes *et al*., 2009). The available nutrients in wheat bran remain surrounded by firm cell. Thus fungal hyphae that fail to penetrate deep inside would not able to draw up the nutrients. Though, wheat and rice straw contain similar proportion of hemicellulose but latter supported least production due to lignin and silica content which masks the hemicellulose, thereby decreasing accessibility of xylan. Xylanase production is affected by the type of xylan, particularly the extent of branching, the type and frequency of side groups on the xylan backbone and the degree of polymerization of the xylan molecule (Jain, 1995) while crude lignocellulosics serve as cheap and effective substrate but co-production of cellulases makes it difficult to realise cellulase-free xylanase. A 2% level of wheat bran supported maximal xylanase production. The improved synthesis of xylanase is affected by the type and concentration of the nitrogen source. Yeast extract served as the best nitrogen source. 1.5% level significantly increased the yield comparable to that supported by 2% wheat bran. Yeast extract has always been a universal choice along with wheat bran for maximal xylanase production (Rawat and Johri, 2004). Supplementation of Ca^{2+} enhanced the enzyme production while tween-80, triton X-100, SDS and EDTA was either ineffective or inhibited enzyme production. Reports of strong inhibition of xylanase activity by $CuSO_4$, EDTA and SDS are available in literature (Gomes *et al*., 1994).

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