RESEARCH PAPER | Botany | Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

Production of Xylanase From *Thermomyceslanuginosusby* Solid State Fermentation

KEYWORDS: Solid state fermentation, Xylanase, Thermomyceslanuginosus, Wheat bran, Yeast extract.

Seema Rawat

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

ABSTRACT *Solid state fermentation (SSF) has more potential for production of enzymes especially by fungi. The present research work aimed at optimization of culture conditions for enhanced production of xylanase by Thermomyceslanuginosus by solid state fermentation. The maximum production was observed at 400C (145.64±4.56 U/g solid) and least at 600C (10.50±2.15 U/g solid).Maximum xylanase production was observed after 96h of incubation (150.0±5.67 U/g solid) and at pH 6.5 (150.0±2.67 U/g solid) and at 1.5% wheat bran (165.10±3.10 U/g solid). Maximum xylanase production was observed on yeast extract (165.10±3.10 U/g solid) and least on potassium nitrate (30.24±3.12 U/g solid).Maximum xylanase production was observed on 1.5% yeast extract (180.10±3.10U/g solid). All additives and detergents decrease the activity of enzyme. Maximum decrease was observed when tween-80 was added (80.25±2.50 U/g solid).Ca2+ and Mn2+ were found to enhance xylanase production while Mg2+, Fe2+ and Cu2+ were found to decrease xylanase production.*

1. Introduction

Xylanase hydrolyses β-1,4 glycosidic linkages between D-xylanopyranose units of xylan, a heteropolysaccharide with substitutions like, acetylation at the C-2 or C-3 of the xylose units; α-1,2- linked glucuronic or 4-O-methylglucuronic acid groups; α-1,3- linked arabinofuranose units and ferulic or coumaric acids esterified to C-5 of arabinose (Coughlan, 1992).The complexity of hydrolysis of xylan, a heterogeneous substrate, to its constituent sugars is reflected by the synergistic action of complement of main-chain and side-chain cleaving enzymes. Solid state fermentation is considered to be more advantageous as compared to submerged fermentation like production of high concentration of products, requirement of simple equipment, cheaper in cost etc (Moo-Young, 1983; Raimbault, 1989; Doelleet al., 1992; Miendalet al., 2011). Agro-residues like wheat bran, wheat straw, sugarcane bagasse, wheat flour, corn flour, mustard oil cake etc. serves as the best substrate for SSF. The substrates may sometime require pre or post treatment to make them ideal for microbial growth (Cabello and Conde, 1985).The fungal cultures produce various hydrolytic enzymes to utilize the agro-residues for their growth. The present work was focused on optimization of culture conditions for enhanced production of xylanase, by *Thermomyceslanuginosus* which is a thermophilic mould, using solid state fermentation.

2. Materials and Methods

2.1 Inoculum

Thermomyceslanuginosus 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 Solid –state fermentation (LSF)

Erlenmeyer flasks (250 ml) containing 10 g wheat bran and 20 ml media (gl^{-1} $MgSO_4.7H_2O$, 0.5; K_2HPO_4, 1.0; yeast extract, 1.0 and peptone, 2.0) were inoculated with 2 discs (4.0 mm dia., 0.2 mm thick) of 4-day old fungal culture and incubated at 50^{0}C for different time interval ranging from 24 h to 120 h. The flasks were intermittently tapped gently during incubation.

2.3 Extraction of crude enzyme

Flasks were removed after every 24 h and 100 ml of phosphate buffer (0.1 M, pH 6.0) containing 0.1% Tween-80 was added to the fermented mixture. Flasks were placed on rotary shaker for 1 h at 180 rpm and then left overnight under refrigeration to effect the release of any bound enzyme. The contents were centrifuged at 8000 rpm for 15 min and the supernatant was used as crude enzyme.

2.4 Enzyme assay

Xylanase activity was determined using birchwoodxylan as substrate. The reaction mixture, containing 0.5 ml of 1% birchwoodxylan 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.

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

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 (145.64±4.56 U/g solid) and least at 60^{0}C (10.50±2.15 U/g solid).

Table 1: Effect of temperature on xylanase production by *Thermomyceslanuginosus*

Temperature (^{0}C)	Activity (U/g solid)
30	30.16±1.78
35	72.10±2.28
40	150.0±5.67
45	145.64±4.56
50	95.10±4.21
55	40.27±2.26
60	10.50±2.15

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 (150.0±5.67 U/g solid) and no production was observed after 12h of incubation.

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

Incubation period (h)	Activity (U/ml)
12	-
24	15.52±2.96
36	35.08±3.05
48	55.91±3.31
60	76.48±2.26
72	100.19±1.98
84	125.49±2.98
96	150.0±5.67
108	100.54±2.56
120	85.32±2.98

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

3.3 Effect of pH on xylanase production
Maximum xylanase production was observed at pH 6.5 (150.0±2.67 U/g solid) and no production was observed at pH 3.0, 3.5 and 10.0.

Table 3: Effect of pH on xylanase production by *Thermomyceslanuginosus*

pH	Activity (U/g solid)
3.0	-
3.5	-
4.0	12.46±1.79
4.5	29.19±2.31
5.0	45.20±3.56
5.5	75.21±1.46
6.0	110.21±2.12
6.5	150.0±2.67
7.0	136.27±2.32
7.5	110.19±1.42
8.0	76.47±2.10
8.5	55.21±2.32
9.0	15.12±2.85
10.0	-

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

3.4 Selection of best carbon source for enzyme production
Maximum production was observed when wheat bran was used as substrate (150.0±2.67 U/g solid) and least when rice straw was used as substrate (117.26±4.20 U/g solid).

Table4:Effect of carbon source on xylanase production by *Thermomyceslanuginosus*

Carbon Source	Activity (U/g solid)
Wheat straw	124.56±2.67
Wheat bran	150.0±2.67
Sugarcane bagasse	135.67±3.10
Rice straw	117.26±4.20

Incubation temp.- 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 (165.10±3.10 U/g solid) and least at 3.0% wheat bran (95.12±2.10 U/g solid).

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

Level (%)	Activity (U/g solid)
0.5	121.12±3.12
1.0	150.0±2.67
1.5	165.10±3.10
2.0	140.35±2.20
2.5	110.13±3.10
3.0	95.12±2.10

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 (165.10±3.10 U/g solid) and least on potassium nitrate (30.24±3.12 U/g solid).

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

Nitrogen source (%)	Activity (U/g solid)
Yeast extract	165.10±3.10
L-asparagine	150.21±2.11
Peptone	145.23±3.13
Tryptone	120.12±3.22
Beef extract	115.24±2.50

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

Casein	35.46±3.45
Malt extract	46.42±2.35
$(NH_4)_2HPO_4$	116.55±3.20
$(NH_4)_2SO_4$	115.55±3.20
KNO_2	30.24±3.12

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 (180.10±3.10U/g solid) and least on 3% (86.37±2.21 U/g solid).

Table 7: Effect of different levels of yeast extract on xylanaseby *Thermomyceslanuginosus*

Level (%)	Activity (U/g solid)
0.5	120.45±2.12
1.0	165.10±3.10
1.5	180.10±3.10
2.0	150.24±4.23
2.5	105.13±3.10
3.0	86.37±2.21

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

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 (80.25±2.50 U/g solid).

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

Supplement	Activity (U/g solid)
Control	180.10±3.10
Tween-80 (0.1%)	80.25±2.50
Triton X-100 (0.02%)	100.45±3.50
SDS (0.001%)	120.10±2.20
EDTA (0.01%)	150.25±3.20

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 supplemented into the medium (195.08±2.56 U/g solid) and least when Cu^{2+} was added (114.16±2.20 U/g solid).

Table9: Effect of divalent cations on xylanase production by *Thermomyceslanuginosus*

Supplement (0.01%)	Activity (U/g solid)
Control	180.10±3.10
Ca^{2+}	195.08±2.56
Mn^{2+}	190.23±3.20
Mg^{2+}	140.32± 2.16
Fe^{2+}	126.24± 3.20
Cu^{2+}	114.16±2.20

4. Discussion

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 realisecellulase-free xylanase. Higher enzyme production in SSF than LSF appears to be on account of a more natural environment for growth of compost isolates besides the production of heat generated in SSF which would support good growth of thermophilic moulds(Jain, 1995).

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 Myceliophthorathermophilum (Dubey and Johri, 1987; Gaffney et al., 2009; Simoeset 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.

A 2% level of wheat bran supported maximal xylanase production (49.85 U) and yield (12.58 U mg^{-1}protein). The improved synthesis of xylanase is affected by the type and concentration of the nitrogen source. Yeast extract at 1% level served as the best nitrogen source; 2% level significantly increased the yield comparable to that supportd by 1.5% wheat bran. Yeast extract has always been a universal choice along with wheat bran for maximal xylanase production (Rawat and Johri, In Press). 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).

Thus this work yields the optimized conditions for maximum production of xylanase by *Thermomyceslanuginosusby* SSF.

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

REFERENCE

1. Alams, M.; Gomes, I.; Mohiuddin, G. and Hoq, M.M. 1994. Production and characterisation of thermostablexylanases by Thermomyceslanuginosus and Thermoascusaurantiacus grown on lignocellulose. Enzyme Microb. Technol. 16: 298-302. | 2. Anand, L., Krishnamurthy, S. and Vithayathil, P.J. 1990. Purification and properties of xylanase from the thermophilic fungus, Humicolalanuginosa (Griffon and Maublanc) Bunce. Arch.Biochem. Biophys. 276: 546-553. | 3. Cabello, A. and Conde, J. 1985. Evaluation of newer methods of pretreatment for biological utilization of cellulosic residues. ActaBiotechnologica. 5:191-196. | 4. Cesar, T. and Mrsa, V. 1996. Purification and properties of the xylanase produced by Thermomyceslanuginosus. Enzyme Microb. Technol. 19: 289-296. | 5. Chadha, B.S.; Jaswinder, K.; Rubinder, K.; Saini, H.S. and Singh, S. 1999. Xylanase production by Thermomyceslanuginosus wild and mutant strains. World J.Microbiol.Biotechnol. 15: 195-198. | 6. Coughlan, M.P. 1992. Towards an understanding of the mechanism of main chain- hydrolyzing xylanases. In: Xylans and Xylanases (Eds: Visser, J., Beldman, G., Kusters-van Someren, M.A. and Voragen, A.G.J.). Elsevier, Amsterdam. 111-139. | 7. Coughlan, M.P. and Hazlewood, G.P. 1993. β-1,4-D- Xylan-degrading enzyme system, biochemistry, molecular biology and applications. Biotechnol.Appl.Biochem. 17:259-289. | 8. deAlmedie, E. M.; de Lourdes, M.; Polizeli, T.M.; Terenzi, H.F. and Jorge, J.A. 1995. Purification and biochemical characterization of β-xylosidase from Humicolagriseavar. thermoidea.FEMS MicrobiolLett. 130:171-176. | 9. Deschamps, F., Raimbault, M. and Senez, J.C. 1982. Solid state fermentation in the development of agro-food by-products. Industry and Environmn. 5:27-30. | 10. Doelle, H.W., Mitchell, D.A. and Rolz, C.E. 1992. Solid substrate cultivation. Elsiever Science Publications Limited, New York, 466 p. | 11. Dubey, A.K. and Johri, B.N. 1987. Xylanolytic activity of thermophilic Sporotrichumsp. and Myceliophthorathermophilum. Proc. Ind. Acad. Sci. 97: 247-255. | 12. Dusterhoft, E.M.; Linssen, V.A.J.M.; Voragen, A.G.J. and Beldman, G. 1997. Purification, characterization and properties of two xylanases from Humicolainsolens. Enzyme Microb. Technol. 20: 437-445. | 13. Gaffney, M., Doyle, S. and Murphy, R. 2009. Optimization of xylanase production by Thermomyceslanuginosus in solid state fermentation. BioSci. Biotechnol. Biochem. 73: 2640-2644. | 14. Grajek, W. 1987. Production of D-xylanases by thermophilic fungi using different methods of culture. Biotechnol. Lett. 9:353-356. | 15. Gomes, D. J.; Gomes, I. and Steiner, W. 1994. Factors influencing the induction of endo-xylanase by Thermoascusaurantiacus. J. Biotechnol. 33: 37-94. | 16. Jain, A. 1995. Production of xylanase by thermophilic Melanocarpusalbomyces IIS-68. Process Biochem. 30: 705-709. | 17. Kitpreechavanich, V.; Hayashi, M. and Nagai, S. 1984. Purification and properties of endo-1,4-β-xylanase from Humicolalanuginosa. J. Ferment. Technol. 5: 415-420. | 18. Kuhad, R.C.; Singh, A. and Eriksson, K. E. L. 1997. Microorganisms and enzymes involved in the degradation of plant fiber cell walls. In: Adv. Biochem. Engg. Biotechnol. (Ed: K.E.L Eriksson). Springer-Verlag, Berlin, Heidelberg, New York, 45-127. | 19. Miendal, B.S., Idil, A. and Umar, A. 2011. Microbiological features of solid state fermentation and its applications- An overview. Res. Biotechnol., 2: 21-26. | 20. Miller, G.L. 1959. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428. | 21. Moo-Young, M., Moriera, A.R. and Tengerdy, R.P. 1983. Principles of solid state fermentation. In: The filamentous fungi, Vol. 4, Fungal Biotechnology (Eds. Smith J.E, Berry D.R & Kristiansen B.). Edward Arnold Publishers, London, pp. 117-144. | 22. Prabhu, K.A. and Maheshwari, R. 1999. Biochemical properties of xylanase from a thermophilic fungus, Melanocarpusalbomyces, and their action on plant cell walls. J. Biosci. 24: 461-476. | 23. Raimbault, M. 1998. General and microbiological aspects of solid substrate fermentation. Electronic J. Biotechnol. 1: 1-15. | 24. Rawat, S. and Johri, B.N. 2002. Ecology and structural diversity of thermophilic fungi. In: Frontiers of Fungal Diversity in India (Eds: G.P. Rao, C. Manoharachari, D.J. Bhat, R.C. Rajak, T.N. Lakhanpal). Int. Book Distributing Co. Lucknow, India. 205-232. | 25. Rawat, S. and Johri, B.N. 2004. Xylanases of thermophilic moulds and their application potential. In: Handbook of Fungal Biotechnology. (Ed.: D. K. Arora). Marcel Dekker Inc. New York, USA. | 26. Simoes, M.L.G., Tornisielo, S.M.T. and Tapia, D.M.T. 2009. Screening of culture condition for xylanase production by filamentous fungi. Afr. J. Biotechnol. 8: 6317-6326. | 27. Su, Y., Zhang, X., Hou, Z., Zhu, X., Guo, X. and Ling, P. 2011. Improvement of xylanase production by thermophilic fungus Thermomyceslanuginosus SDYKY-1 using response surface methodology. J. Biotechnol. 28: 40-46. |