Effect of Xylan on XylanaseProduction by *Bacillus pumilus* Under Submerged Fermentation

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Abstract

Xylanase production by the alkalophilic *Bacillus pumilus* was improved under submerged fermentation when commercial Birchwood xylan was used as the substrate. Initially the medium containing commercial Birchwood xylan 20gL^{-1} at pH 8.5 was used. When the commercial Birchwood xylan concentration was 5gL^{-1} highestxylanase activity [61.3(±0.96) UmL^{-1}] was obtained at 24 h. The media substituted with raw materials of local carbon sources and considerable amount of xylanase was produced in the media containing corn cob[24.9(±0.99) UmL^{-1}], corn hull [18.2(±0.92) UmL^{-1}] and rice straw [21.5(±0.86) UmL^{-1}] and lowest amount of activity was obtained with rice bran [4.7(±0.98) UmL^{-1}]. Xylan was extracted from local carbon source such as corncob, corn hull and rice straw and these were used as the carbon source in the media and Birchwood xylan was used as the control. The highest xylanase activity was obtained in the medium with Birchwood xylan [(62.6(±0.89) UmL^{-1})] than in the media which contained the xylan (5gL^{-1}) extracted from corncob [29.8(±0.97) UmL^{-1}] corn hull [20.2(±0.99) UmL^{-1}] and rice straw [17.6(±0.98) UmL^{-1}]. Due to the amount of xylose present in the xylan, the highest activity was obtained with Birch wood xylan. Based on this study the xylanase production from *B.pumilus* was better with commercial Birchwood xylan than that extracted from different local sources

Key words: Corncob, corn hull, Rice straw, xylanase and Xylan

Introduction

Xylanase has attached special attention due to its potential applications in many processing industries (Lemoset al., 2001; Wong et al., 2000). The use of purified xylan as a substrate for bioconversion into xylanase increases the cost of enzyme production. Consequently, for commercial applications, there have been attempts to develop to produce xylanase in high quantities from inexpensive substrate. *Thermomyces lanuginosus* is known to produce high levels of

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cellulose-free xylanase in submerged culture using corncob as carbon source (Sing et al., 2000; Puchartet al., 1999). However enzyme production is related to the type and concentration of carbon and nitrogen sources. Several different strategies were applied in order to enhance the enzyme production by various microorganisms (Gupta et al., 2000;Fenget al., 2003). In general, medium optimization technique is used to increase the enzyme production. This work is integrated to improve the medium for better xylanase production by using different agricultural wastes as the main carbon sources, by *Bacillus pumilus* under submerged fermentation.

Materials and Methods

Materials

Birchwood xylan (Roth, Germany) from standard source were used. Corncob, corn hull, rice straw and rice bran were sun dried and powdered in a domestic grinder.

Microbial strain

In this study xylanase producing bacterial strain *Bacillus pumilus* isolated from corncob decaying soil was used for enzyme production (Jesuthasanet al., 2010).

Culture Media and Production of xylanase

The Xylan Nutrient Agar plates and slants containing (gL^{-1}) nutrient agar, 28.0 and Birchwood xylan, 20.0 at pH 8.5 was used for the storage of the isolates and incubated at 40^{o}C for 24 h.

The activation medium contained (gL^{-1}) xylan, 20.0 and nutrient broth, 25.0 at pH8.5. The bacterial colonies grown on the slant were transferred to 100mL conical flask containing 10mL of activation medium (1 loop/10mL) and incubated in reciprocal shaker water bath at 45^{o}C and at pH 8.5, 120 rpm for 18 h.

Fermentation medium contained (gL^{-1}) xylan, 20.0; peptone, 2.0; yeast extract, 2.5; $CaCl_2.2H_2O$, 0.005; $MgCl_2.6H_2O$, 0.005; $FeCl_3$, 0.005; K_2HPO_4, 2.5; KH_2PO_4, 1.0; NaCl, 0.1 and $(NH_4)2SO_4$, 2.0 at 8.5 pH. Fermentation medium was inoculated with the activated culture (20%, v/v) and incubation was carried out at 45$_{o}$C for 32h. Enzyme assay was carried out after the removal of the cells by centrifugation at 3000 rpm for 20 min.

In shaker flask experiments, media volume to shaker flask volume ratio was maintained as 1:10. All the experiments were carried out in triplicate.

Xylanase activity assay

Assay mixture consisted of 0.25 mL of diluted enzyme solution and 0.25 mL of 20 gL^{-1} xylan in 0.05M Tris-HCl buffer, pH 8.4. After incubation at 60^{o}C for 4 min, the increase in reducing sugars was determined by DNS method (Miller, 1959) with xylose as the standard.

One unit of xylanase activity is defined as the amount of enzyme that releases one µmol of reducing sugar equivalent to xylose per minute at 60°C and pH 8.4 with 20 gL^{-1} xylan.

Effect of xylan to improve xylanase titre

Effect of commercial Birchwoodxylan

Fermentation media with different amounts of commercial Birchwood xylan (1-30gL^{-1}) were inoculated with activated *Bacillus pumilus*, incubated at 45°C and 120rpm and xylanase production was monitored.

Effect of xylan from different agricultural waste materials

Effect of different xylan containing raw materials

Local carbon sources (10gL-1) such as grains removed dried corncob powder, corn hull powder, rice straw powder and rice bran powder substituted to optimized amount of commercial Birchwood xylanin the fermentation medium and xylanase production was studied.

Extraction of xylan from local carbon sources

Lignocellulose materials in the corncob, corn hull and rice straw were extracted using a modified method described by Balasubramaniam (1976).

The yield (%) was calculated using following equation.

$$\text{The yield of xylan (X)} = \frac{\text{Amount of extract obtained (kg)}}{\text{Corncob used (kg)}} \times 100$$

Effect of xylanextracted from different sources

Xylan extracted from corncob, corn hull and rice straw (5gL^{-1}) in fermentation medium was used for xylanase production. Optimized amount of commercial xylan from Birchwood in the fermentation was used as the control.

Effect of xylan extracted from corncob

Fermentation media with different amounts of xylan(5-20gL-1) extracted from corncob were used for xylanase production.

RESULTS

Effect of commercial Birchwood xylan

Concentration of Birchwood xylan in the fermentation medium was increased from 1 to 5gL^{-1} and the activity of xylanase was increased from 58.7 to 61.3UmL^{-1}. Further increase in the concentration of xylan from 5 to 30gL^{-1} has decreased the xylanase activity by 36.3UmL^{-1}. With all the xylanase concentrations highest xylanase activity was produced at 24h (Figure 1).

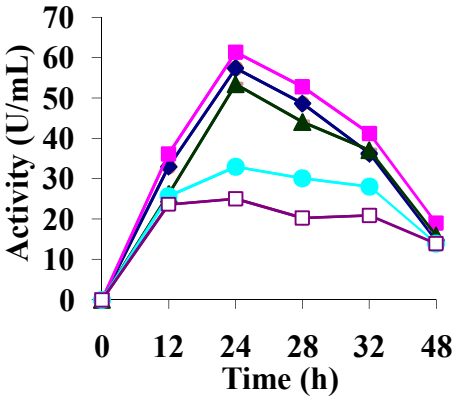

Figure 1: Xylanase produced by B.pumilus in fermentation medium having different concentration of commercial Birchwood xylan such as ( ) 3.0gL^{-1}, ( ) 5.0gL^{-1}, ( ) 10.0gL^{-1}, ( ) 20.0gL^{-1} and ( ) 30.0gL^{-1} at 45^{o}C and pH 8.5.

In the control medium containing 20gL^{-1} xylan,32.9UmL^{-1} xylanaseactivity was obtained. Hence a 4 fold decrease in xylan from 20 to 5gL^{-1} has increased the xylanase activity by 1.8 times. Therefore 5gL^{-1} of commercial Birchwood xylan was chosen for the further studies.

Effect of local carbon sources

At 24h, highestxylanase activity was produced in the control medium which contained commercial Birchwood xylan (60.2UmL^{-1}). Among the local xylan sources, xylanase production was highest in the media with corncob [24.9(±0.99) UmL^{-1}] followed with corn hull [18.2(±0.92) UmL^{-1}] and rice straw [21.5(±0.86) UmL^{-1}] and negligible amount of xylanase was produced in the medium containing rice bran [4.7(±0.98) UmL^{-1}] (Figure 2).With all the local xylan sources highest xylanase activity was obtained at 12h.

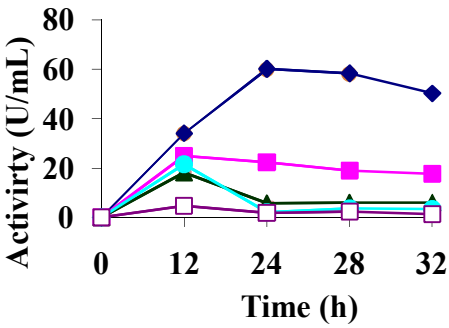

Figure 2: Activity of xylanase in fermentation medium having commercial Birchwood xylan ( ) and different local carbon sources such as ( ) corncob, ( ) corn hull, ( ) rice straw and ( ) rice bran at 45^{o}C and pH 8.5.

Extraction of xylan from local carbon sources

From 1.0kg of corncob, corn hull and rice straw, 61.7(±1.29), 51.32(±1.24) and 41.6(±1.17) g of xylan was extracted.

Effect of xylan extracted from different sources on xylanase production

As highest xylanase activity was produced in the medium with 5gL-1 commercial Birchwood xylan (62.6(±0.89) UmL^{-1}),5gL^{-1} xylan extracted from different sources were added in the fermentation medium instead of Birchwood xylan. Xylanase activity obtained in the media which contained 5gL-1xylan extracted from corncob, corn hull and rice straw were 29.8(±0.97), 20.2(±0.99) and 17.6(±0.98)

UmL^{-1} respectively (Figure 3). As xylan extracted from corncob gave highest xylanase activity among the xylan extracted from local sources it was selected for further studies.

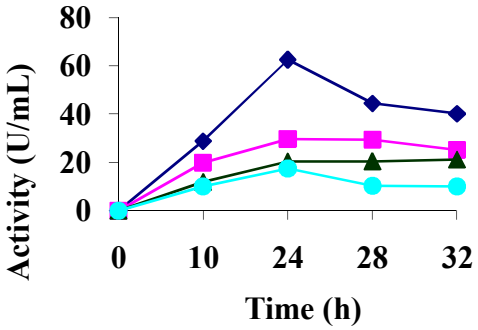

Figure 3: Activity of xylanase in fermentation medium having different xylans such as (◆) commercial Birchwood xylan; (■)xylan extracted from corncob xylan; (▲) xylanextracted corn hull and (●) xylanextracted rice straw at 45oC and pH 8.5.

Effect of xylanextracted from corncob

When *B.pumilus* was grown in media with different concentrations of xylan extracted from corncob, xylanase activity was highest [27.6(±0.88) UmL^{-1}] at 10h, in the medium containing 7.5gL^{-1}xylan extracted from corncob (Figure 4). When the concentration of xylan extracted from corncob was increased from 2.5 to 7.5, the xylanase activity was increased from 25.75 (±1.09) to 31.3(±1.01) UmL^{-1}, while when the concentration was increased from 7.5 to 20gL^{-1} the xylanase activity was decreased by 13.4UmL^{-1}. Higher amount of (68.2 UmL^{-1}) xylanase activity was obtained in control medium which contained commercial Birchwood xylan.

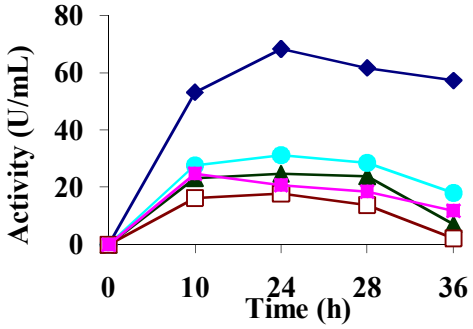

Figure 4: *Activity of xylanase in fermentation medium having different concentration of xylan extracted from corncob (▲) 5gL^{-1}, (●) 7.5gL^{-1}, (■) 10gL^{-1}; (□) 20gL^{-1} and (◆) commercial xylan at 45°C and pH 8.5.*

DISCUSSION

Commercial xylan is expensive for large-scale production of xylanases and lignocellulosic materials can be used as cost-effective substrates for xylanase production (Haltrichet al., 1996; Beg et al.,2000). Therefore in the following experiments locally available xylan sources such as corncob, corn hull, rice straw and rice bran powders were used as carbon sources for xylanase production.

Several potential biomass resources, corncob (Koga and Fujikawa, 1985; Pelleninet al., 1991) and corn hull (Kurakakeet al., 2005) have been frequently reported in the literature as the most promising materials for

xylooligosaccharide production (Koga and Fujikawa, 1985;Pelleninet al., 1991). Rice bran was used for the production of β-xylanase (Wang et al., 2003) as the sole carbon source by *Streptomyces actuosus* A-151.

The difference in the xylanase production in medium with different xylan sources could be decreased due to the impurities and variation in their concentration in the raw materials. Further the high xylanase production with commercial source could be due to its purity. To find the exact reason for the reduction of xylanase production in the medium with the local sources, the xylan in the local sources were extracted and used for xylanase production. When different carbon source were used xylanase production was least in the medium which contained rice bran. Therefore corncob, corn hull and rice straw were used for the extraction of xylan and rice bran was excluded from the studies.

The highest enzyme production with Birch wood xylan may due to the amount of xylose present in the xylan. The amount of xylose from Birch wood xylan is 94.1% (Li et al., 2000), while based on the composition analysis studies the corn cob, corn hull and rice straw xylan contained 69.3, 57.9 and 46.5g% of xylose. Xylanase production by Bacillus laterosporus grown in medium containing commercial Birchwood xylan was 2.2 fold higher than that in the medium which contained xylan extracted from corncob (Chinniahet al., 2005).

Since the extracted xylans did not give better xylanase activity as the commercial Birchwood xylan, for the following studies, commercial Birchwood xylan was used as the carbon source.

CONCLUSION

Xylanase production was improved by commercial Birchwood xylan than the xylan extracted from corncob, corn hull, rice straw and rice bran. Among the xylan extracted from different sources, that extracted from corncob gave better xylanase production than other extracted xylans.

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REFERENCES

Q.K. Beg, B. Bhushan,M.Kapoor, G.S. Hoondal,Ind. Microbiol.andBiotechnol.24, 396-402 (2000).

G.l. Miller, Ana.Chem. 3,426-428 (1959).

J.L.S. Lemos, M.C.A. Fontes, andN.J. Pereire, Appl.Biochem.andBiotechnol.91,681-689 (2001).

K.K.K. Wong, C.S. James, andS.H. Campion, J. pulp paper Sci., 26, 377-383 (2000).

S.Jesuthasan, S.Balakumar, andV. Arasaratnam,Proceedings of Jaffna Science association.17, 22(2010).

K.Balasubramanium, J. of Food Sci.41, 1370-1374(1976).

K.Koga, andS.Fujikawa, 'Xylooligosaccharides', In: Oligosaccharides, 3, 130-143 (1985).

P.Pallenin, M. Gosselin, J.Lepoutre, E.Samain, andP.Deheire, Enzyme and Microbial Technol.13,617-621 (1991).

V. Puchart, P. Katapodis, P.Biely, L. Kremnicky, P. Christakopoulos, M.Vrsanska, D. Kekos, B.J. Marcis, andM.K. Bhat, EnzymeMicrobiol.Technol.24,355–361 (1999).

D.Haltrich, B.Nidetzky, K.D. Kulbe,W. Steiner and S.Zupancic,BioresoueceTechnol.58, 137-161(1996).

A. Gessese, and G. Mamo,Enzyme Microbiol.andTechnol.25, 68-72(1999).

E.T. Chivero, A.N. Mutukumira, andR. Zvauya, Food Chem.72,179–185 (2001).

J.X. Heck, L.H.B. Soares, P.F. Hertz, andM.A.Z. Ayub, Biochemcal Eng.32, 179–184 (2006).

S. L.Wang,Y. H.Yen,I. L.Shih,A.C.Chang,W. T.Chang,and W. Wu,Enzyme Microbiol.Technol.33,917–25 (2003).

K.Li, , P. Azadi, R. Collins, J. Tolan, J.S.Kim, and K. E. L. Eriksson, Enzyme Microbiol.Technol.27, 89-94 (2000).

M.Kurakake, K.Ouchi, W. Kisaka, and T. Komaki, J. of Appl.Glycoscience.52 (3),281-285(2005).

M. Chinniah, M.Phil. Thesis, University of Jaffna, Sri Lanka(2005).

P. Feng, Z.He, S. L.Ong, J.Hu, Z.Zhang, and W.J. Ng, Enzyme Microbiol.Technol.32, 282-289(2003).

S.Singh,B. Pillay,andB. A. Prior, Enzyme Microbiol.Technol.26,502-508 (2000).

S.Gupta, B.Bhushan, G. S.Hoondal, J. Appl. Microbiol..88,325–334 (2000).