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Optimization of Xylanase Production from *Paenibacillus* sp. DG-22

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Abstract

Investigations were carried out to optimize the culture conditions for the production of xylanase by *Paenibacillus* sp. DG-22, a moderately thermophilic bacterium isolated from timber yard soil. Xylanase production showed a cell growth associated profile. Xylanase activity was found only in the culture supernatant, while β-xylosidase activity was mainly associated with the cells. The formation of xylanase activity was induced by xylan and repressed by glucose and xylose. The production profile of xylanase was examined with various commercial xylan and maximum yield was achieved with 0.1~0.5% birchwood xylan. Among various nitrogen sources tested, yeast extract was optimal for the production of xylanase. The xylanase activity was inhibited by Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+} and Mn^{2+} ions while Ca^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+} ions and DTT stimulated xylanase activity. Mercury (II) ion at 5 mM concentration abolished all the xylanase activity. The predominant products of xylan-hydrolysate were xylobiose, xylotriose, and higher xylooligosaccharides, indicating that the enzyme was an endoxylanase.

Key words – thermostable xylanase, *Penibacillus* sp. culture conditions

Introduction

Xylan constitutes 20~30% of the weight of wood and agricultural wastes. It is the second most abundant polysaccharide after cellulose found in plant cell walls. Thus xylan is a significant renewable biomass which can be utilized as a substrate for the preparation of many useful products such as fuels, solvents, and chemicals. Xylan is a heteropolymer with a backbone of β(1→4)-linked xylosyl residues and branches of neutral or uronic monosaccharides and oligosaccharides[4]. Biodegradation of xylan requires the combined action of several xylanolytic enzymes, among which xylanases (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) and β-xylosidase (β-D-xyloside xylohydrolase, EC 3.2.1.37) are crucial for xylan depolymerization. Endoxylanases catalyze the hydrolysis of xylan to xylo-oligosaccharides and xylose, while β-xylosidases release xylose residues from the nonreducing ends of xylo-oligosaccharides. These xylanolytic enzymes are produced by various microorganisms[4,8,24].

In recent years, xylanases have received considerable research interest due to their potential industrial applications. They could be used in bioconversion of lignocellulosics to fuels, chemicals, pentose production, fruit juice clarification, in improving rumen digestion and in biobleaching of pulp[3,21]. The application of xylanase for bleaching of kraft pulp has received great attention recently because of environmental concerns. The pretreatment of paper pulps with xylanases can reduce the amount of chlorine used for bleaching in the brightening process[23]. The enzyme used for the purpose of bio-

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bleaching should be active at higher temperature, thermostable, alkalophilic and cellulase free xylanase. Recently the interest in thermostable enzymes has increased and thermostability has become a desirable property of enzymes used in many industrial applications. The advantages of thermostable enzymes in industrial processes include reduced risk of contamination, increased solubility of the substrate, increased diffusion rates and higher stability of enzymes against denaturing agents and proteolytic enzymes[6,25].

In a previous report, the isolation of a moderately thermophilic *Paenibacillus* sp. strain DG-22 from a timber yard soil in Kyungju, Korea have been described[11]. This strain produces significant amounts of thermostable xylanase without cellulase activity. The objectives of the present investigation were to evaluate the effects of nutritional factors on the production of xylanase from *Paenibacillus* sp. strain DG-22 and to optimize a fermentation process in order to assist in purification and biochemical studies.

Materials and Methods

Bacterial strain and culture conditions

Moderately thermophilic *Paenibacillus* sp. DG-22 was used in this investigation[11]. Enzyme production was studied in Luria-Bertani (LB) medium or M9 minimal medium[19] supplemented with 0.2% xylan. Cultivation of the organism was performed in 250 ml Erlenmeyer flasks containing 50 ml of medium. Flasks were shaken on an orbital shaker at 150 rpm at 45℃. After growth, cells were removed by centrifugation and the supernatant was used as the source of crude enzyme.

Chemicals

Commercial xylans (birchwood xylan, beechwood xylan, oatspelt xylan), peptone (from meat and soybean) and corn steep liquor were purchased from Sigma (St. Louis, MO., U.S.A.). Beef extract, yeast extract, Bactopeptone, proteose peptone and tryptone were purchased from Difco (Sparks, MD, U.S.A.). Xylobiose, xylotriose and xylotetraose were purchased from MegaZyme (North Rocks, Australia). All other chemicals were of analytical grade and purchased from Sigma unless otherwise stated.

Localization of xylanase and β-xylosidase

The bacterial cells were grown for 16 h at 45℃ in an orbital shaker in 250 ml flasks containing 50 ml of LB medium supplemented with 0.2% birchwood xylan. The cultures were harvested by centrifugation (4,000 $\times$ g, 4℃, 15 min) and the supernatants were recovered for enzymatic activities assays. Cells were washed twice with 0.85% NaCl and resuspended in the same volume of 50 mM sodium acetate buffer (pH 5.0). The suspension was sonicated for 10 min by cooling in an ice bath with a sonifier (model VCX400, Sonics & Materials Inc., Dianburg, CT, U.S.A.). Xylanase and β-xylosidase activities were assayed in the supernatant solutions and in homogenates of whole cells. The experiment was done in duplicate.

Xylanase production by *Paenibacillus* sp. DG-22

For observation on the time course of extracellular xylanase production, cells were cultured at 45℃ and samples were removed at 1 h intervals for enzyme assays. The culture supernatants were collected by the low centrifugation as described above and then appropriately diluted culture supernatants were used for enzyme assays. Bacterial growth was measured by spectrometric method at 600 nm (Pharmacia, model ultrospec 2000).

Enzyme activity assays and protein determination

Xylanase activity was determined by using soluble birchwood xylan (1%, w/v) in 50 mM sodium acetate buffer, pH 5.0, prepared according to the method described by Bailey *et al.*[1]. The amount of reducing sugar liberated was determined by the 3,5-dinitrosalicylic acid

(DNS) method[13] with D-xylose as standard. The assay was carried out at 60℃ for 10 min. One unit of xylanase activity was defined as the amount of enzyme which produced 1 μmol of xylose equivalent per min under the assay conditions. β-Xylosidase activity was measured with *p*-nitrophenyl-β-D-xylopyranoside (2 $mg.ml^{-1}$) as a substrate. A 0.5 ml of 1 M sodium acetate buffer (pH 5.0) was incubated with 0.25 ml of the appropriately diluted enzyme solution and 0.25 ml of substrate solution at 60℃ for 10 min. The reaction was stopped by adding 2.0 ml of 1 M Na_2CO_3 and the absorbance was measured at 420 nm. One unit of β-xylosidase was defined as the amount of enzyme which released 1 μmol *p*-nitrophenol in 1 min. The amount of protein in each sample was determined according to the microassay of Bradford[5] using a standard curve of bovine serum albumin, fraction V (Sigma).

Influence of xylan on enzyme production

The effect of xylan on the formation of xylanase was studied by growing *Paenibacillus* sp. DG-22 under different concentrations of birchwood xylan. The strain was grown in 250 ml culture flasks containing 50 ml M9-yeast extract (0.05%) medium supplemented with different concentrations of birchwood xylan. The inoculated flasks (in duplicates) were incubated at 45℃ in orbital shaker. Cells were harvested in the stationary phase and culture supernatants were collected for crude enzyme sources.

Effect of various additives on the xylanase activity

The influence of different metal ions, EDTA and protein disulphide reducing agents, dithiothreitol (DTT), on xylanase activity were evaluated by DNS assay. The crude enzyme solution was diluted with each of these compounds to give final concentrations of 1 mM and 5 mM. These mixtures were incubated at 30℃ for 10 min. These enzyme/metal ion mixtures were immediately used in the assay for xylanase activity as described above. A control, in which sodium acetate buffer replaced metal ion solutions was set up. The degree of inhibition or stimulation of enzyme activity was expressed as a percentage of the initial activity in the control experiment. The experiment was done in duplicate.

Analysis of xylan hydrolysis products

Xylan hydrolysis reaction was carried out with 10 mg of birchwood xylan and 10 U of crude xylanase in 1 ml of a 50 mM sodium acetate buffer (pH 5.0) at 60℃. The aliquots were periodically withdrawn and xylan hydrolysis products were analyzed by thin-layer chromatography (TLC). A 5 $\mu\ell$ portion of each sample was spotted onto silica gel plate 60 F_{254} (Merck, Darmstadt, Germany) and chromatographed in a solvent system containing n-propanol-ethanol-water (7 : 1 : 2) at room temperature. The sugar spots were detected on the plates by spraying them with 5% sulfuric acid in ethanol, followed by incubation at 110℃ for about 10 min. Xylose (from Sigma), xylobiose, xylotriose, and xylotetraose (from Megazyme) were used as standards.

Results and Discussion

Localization of xylanolytic enzymes

The enzyme activities of xylanase and β-xylosidase were evaluated in both supernatants and whole cell fractions (Table 1). The xylanase activity of exponentially growing *Paenibacillus* sp. DG-22 culture was found main-

Table 1. Localization of the xylanase and β-xylosidase produced by *Paenibacillus* sp. DG-22. Cells were grown for 16 h at 45℃, 150 rpm in shake flasks containing 50 ml LB medium and 0.2% birchwood xylan, pH 6.5

	Xylanase (U/ml)	β-Xylosidase (U/ml)
Supernatant	22.03	24.27
Cells	0.25	107.48

ly in the supernatant solution indicating that xylanase is excreted to the medium. The β-xylosidase activity was primarily cell-associated and less than 20% of the total activity was found in the culture supernatant. The β-xylosidase activity in the culture supernatant may be due to cell lysis and the release of intracellular enzyme into culture medium.

Xylanase production by *Paenibacillus* sp. strain DG-22

A typical growth curve and enzyme production by *Paenibacillus* sp. DG-22 in LB medium containing 0.2% (w/v) birchwood xylan are shown in Fig. 1. There was little xylanase synthesis up to 10 h, after which levels increased continuously accompanying the increase of optical density (OD) which suggests that the xylanase production is growth associated. The xylanase was produced maximally at the end exponential phase and was stable throughout the stationary phase.

Regulation of xylanase production

Substrate specificity of the xylanase was investigated in LB and M9 media supplemented with 0.2% (w/v) different commercial xylans. Among the three commercial xylan used as carbon source, the highest xylanase activity was obtained with birchwood xylan, followed by beechwood xylan and oatspelts xylan (Table 2).

Production of xylanase by *Paenibacillus* sp. DG-22 was studied by growing the organism in LB media containing 0.5% (w/v) of different carbon sources. Production of xylanase was induced by xylan and the production was repressed by glucose or xylose addition (Table 3). Similar results have been reported for many *Bacillus* strains where xylan showed a strong inducing effect[14,18,22], while glucose and xylose repressed the xylanase production[9,20]. These results would suggest that expres-

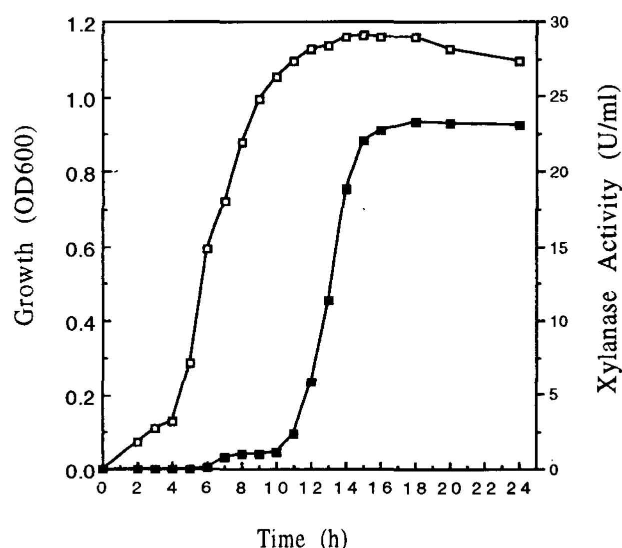

Fig. 1. Production of xylanase during growth of *Paenibacillus* sp. DG-22 at 45℃ in LB medium supplemented with 0.2% (w/v) birchwood xylan (□, growth; ■, xylanase activity in supernatant).

Table 2. Effect of commercial xylans on xylanase production by *Paenibacillus* sp. DG-22

Media	Xylanase activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)
M9 only	0.04	0.007	5.71
M9+beechwood xylan	22.92	0.070	327.43
M9+birchwood xylan	23.30	0.065	358.46
M9+oatspelt xylan	11.86	0.044	269.54
LB only	0.31	0.084	3.69
LB+beechwood xylan	23.42	0.115	203.65
LB+birchwood xylan	23.51	0.108	217.68
LB+oatspelt xylan	19.59	0.095	206.21

Table 3. Xylanase activity of *Paenibacillus* sp. DG-22 grown on LB medium supplemented with various substrates (0.5%, w/v). The means of triplicate measurements and their standard deviations are listed

Substrate	Xylanase activity (Uml^{-1})
None	0.81±0.004
Xylan (birchwood)	21.79±0.082
Xylan+glucose	0.05±0.002
Xylan+xylose	0.07±0.001
Glucose	0.05±0.006
Xylose	0.05±0.005

sion of the xylanase of *Paenibacillus* sp. DG-22 is catabolite repressed.

Effect of nitrogen sources

The effect of various organic nitrogen sources on the production of xylanase by *Paenibacillus* sp. DG-22 are shown in Fig. 2. The M9 minimal medium was supplemented with 0.2% (w/v) birchwood xylan as a sole carbon source and the respective nitrogen sources (0.1%, w/v). After an incubation period of 20 h, xylanase activities were determined in the culture supernatants. Yeast extract, tryptone, beef extract, and corn steep liquor gave rise to higher xylanase production. It was observed that in general organic compounds induced more xylanase production than inorganic ones. Yeast extract gave the best results for the production of xylanase activity. This observation coincides with those in a previous report[17] stating that yeast extract is a critical component of the medium for *B. polymyxa* growth providing a small amount of an essential growth factor. This effect of yeast extract may be also attributed to the increasing concentration of both nitrogen and carbon content in the culture

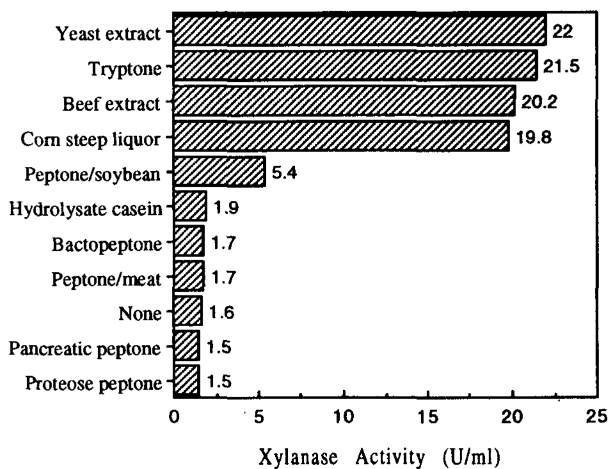

Fig. 2. Effect of various organic nitrogen sources (0.1%, w/v) on xylanase production by *Paenibacillus* sp. DG-22 growing in M9 medium at 45℃ for 20 h. The carbon source was birchwood xylan (0.2%, w/v).

media. Therefore yeast extract was selected as the nitrogen source for further studies.

Effect of substrate concentration on xylanase production

Since the maximum enzyme activity was obtained with birchwood xylan, the influence of its varying concentrations on xylanase production was examined (Fig. 3). The M9 medium was supplemented with different concentrations of birchwood xylan (0~1.5%) and 0.05% yeast extract as a sole nitrogen source. It was observed that xylanase activity was maximum when the substrate was used at 0.1~0.5% level. Xylanase production was characterized by an initial rapid increase in activity at low concentration followed by a steady decline at higher substrate concentration. It has been shown that the presence of decomposition products, such as low molecular weight oligosaccharides present in the substrate, may

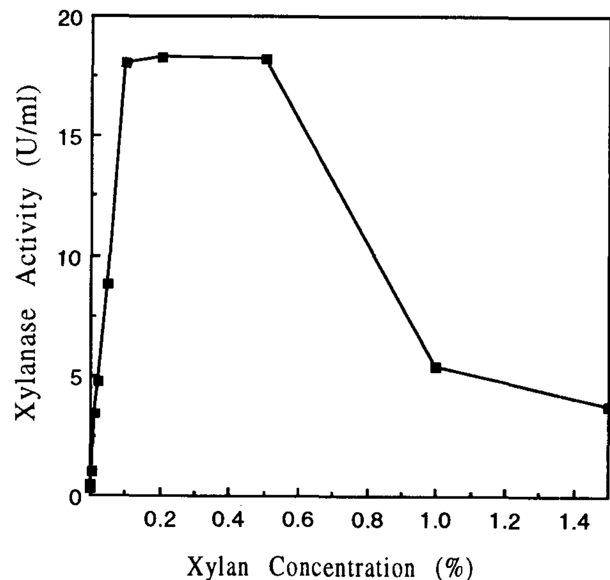

Fig. 3. Efect of xylan concentration (0~1.5%, w/v) on the production of extracellular xylanase in M9-yeast extract (0.05%) medium. The cultures were harvested after 20 h of incubation and cell-free supernatants were assayed for enzyme activities. Data are presented as means of three replicates.

contribute to repression of xylanase production, particularly when high substrate concentrations are used. It is also possible that the xylanase was repressed in a similar fashion with the accumulated hydrolysis products resulting in catabolite repression of enzyme production at higher substrate concentration. The other reason for this may be due to irreversible adsorption of enzyme which was accelerated particularly by the high bulk density of the medium[16].

Effect of various additives on the xylanase activity

The activity of xylanase was measured under the standard assay conditions in the presence of several metals, a metal chelator (EDTA) and protein disulfide reducing agents, dithiothreitol (DTT) at 1 mM and 5 mM (Table 4). Stimulatory effects were obtained with Ca^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+} and DTT. Xylanase activity was reduced by Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+} and Mn^{2+}. Cu^{2+} and Fe^{3+} ions reduced activity to less than 4% at 1 mM, but more than 40% activity was reduced at 5 mM. Hg^{2+} ions completely inhibited the enzyme at 5 mM concentrations. The inhibition of *Paenibacillus* sp. DG-22 xylanase by Hg^{2+}, Cu^{2+}, and Fe^{3+} suggests that these ions are complexing with the acidic amino acid residues at the active site. The effect of EDTA on xylanase activity is unclear. The xylanase was slightly increased by 1 mM EDTA, but the xylanase activity was decreased as the EDTA concentration increased to 5 mM. The enzyme activity was stimulated in the presence of protein disulfide reducing agents, DTT. It seems that there is a relationship between the reduced form of the cysteine residue and the activity of the xylanase.

Table 4. Effects of metal ions, EDTA and DTT on xylanase activity. Enzyme assays were performed as described in Materials and Methods. Relative activity is defined as percentage of residual activity compared to a control

Metal ion	Relative activity (%)	
	1 mM	5 mM
None	100	100
$CaCl_2$	103	104
$CoCl_2$	98	91
$CuCl_2$	99	22
$FeCl_3$	97	56
$HgCl_2$	63	0
KCl	101	102
$MgCl_2$	105	112
$MnCl_2$	95	81
$NiCl_2$	106	108
$ZnCl_2$	104	120
EDTA	106	90
DTT	110	115

Xylan hydrolysis

The hydrolysis products from birchwood xylan were analyzed by silica gel thin-layer chromatography. The enzyme degraded xylan at random, and the end prod-

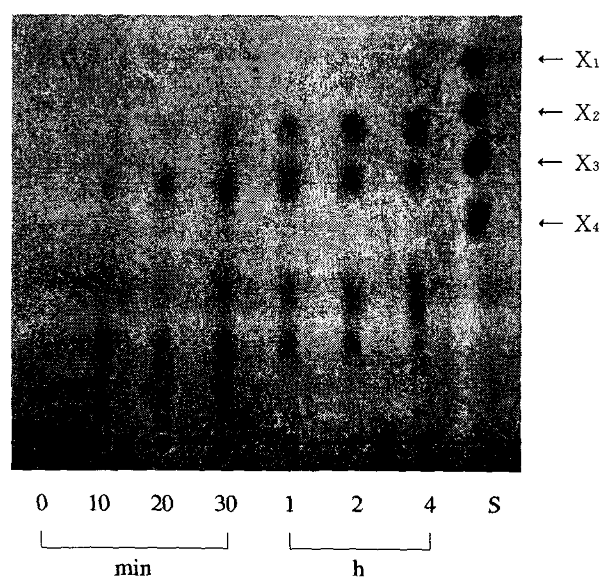

Fig. 4. TLC analysis of xylan hydrolysis products from birchwood xylan. The crude xylanase (10 U) was incubated with 1.0% (w/v) birchwood xylan in 50 mM sodium acetate buffer pH 5.0 at 60℃ for the times indicated. The standards (S) used were xylose (X_1), xylobiose (X_2), xylotriose (X_3) and xylotetraose (X_4).

ucts released were xylobiose, xylotriose, and higher xylooligosacharides. Small amounts of xylose were also detected (Fig. 4). The hydrolysis pattern of xylan during its incubation with a crude preparation of xylanase activity indicated that the enzyme activity mainly responsible for the xylan degradation was an endoxylanase activity. Most *Bacillus* endoxylanases release xylose from xylan[2,15], but the xylose released under prolonged incubation might be due to the β-xylosidase present in the supernatant. Although most β-xylosidase activity was found to be associated with the cells, extracellular β-xylosidase activity was always present to some degree.

Acknowledgment

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References

1. Bailey, M. J., P. Biely and K. Poutanen. 1992. Interlaboratory testing of methods for assay of xylanase activity. *J. Biotechnol.* **23**, 257-270.
2. Bataillon, M., A.-P. Nunes Cardinali, N. Castillon and F. Duchiron. 2000. Purification and characterization of a moderately thermostable xylanase from *Bacillus* sp. strain SPS-0. *Enzyme Microb. Technol.* **26**, 187-192.
3. Beg, Q. K., M. Kapoor, L. Mahajan and G. S. Hoondal. 2001. Microbial xylanases and their industrial applications: a review. *Appl. Microbiol. Biotechnol.* **56**, 326-338.
4. Biely, P. 1985. Microbial xylanolytic systems. *Trends Biotechnol.* **3**, 286-290.
5. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
6. Bruins, M. E., A. E. M. Janssen and R. M. Boom. 2001. Thermozymes and their applications. *Appl. Biochem. Biotechnol.* **90**, 155-186.
7. Coughlan, M. P. and G. P. Hazelwood. 1993. β-1,4-D-xylan-degrading enzyme systems: Biochemistry, molecular biology, and applications. *Biotechnol. Appl. Biochem.* **17**, 259-289.
8. Dekker, R. F. H. and G. N. Richards. 1976. Hemicellulase: their occurrence, purification, properties, and mode of action. *Adv. Carbohydr. Chem. Biochem.* **32**, 277-352.
9. Esteban, R., A. Chordi and T. G. Villa. 1983. Some aspects of a 1,4-β-D-xylanase and β-D-xylosidase secreted by *Bacillus coagulans* strain 26. *FEMS Microbiol. Lett.* **17**, 163-166.
10. Kulkarni, N., A. Shendye and M. Rao. 1999. Molecular and biotechnological aspects of xylanases. *FEMS Microbiol. Rev.* **23**, 411-456.
11. Lee, Y.-E. 2003. Characterization of thermostable xylanases from the newly isolated *Paenibacillus* sp. strain DG-22. Manuscript in preparation.
12. Lee, Y.-E., S. E. Lowe and J. G. Zeikus. 1993. Regulation and characterization of xylanolytic enzymes of *Thermoanaerobacterium saccharolyticum* B6A-RI. *Appl. Environ. Microbiol.* **59**, 763-771.
13. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal. Chem.* **31**, 426-428.
14. Nakamura, S., K. Wakabayashi, R. Nokai, R. Aono and K. Horikoshi. 1993. Production of alkaline xylanase by a newly isolated alkalophilic *Bacillus* sp. strain 41M-1. *World J. Microbiol. Biotechnol.* **9**, 221-224.
15. Nakamura, S., Y. Ishiguro, R. Nakai, K. Wakabayashi, R. Aono and K. Horikoshi. 1995. Purification and characterization of a thermophilic alkaline xylanase from thermoalkaliphilic *Bacillus* sp. strain TAR-1. *J. Mol. Catalysis B: Enzymatic* **1**, 7-15.
16. Pham, P. L., P. Taillandier, M. Delmas and P. Streehaiano. 1998. Production of xylanases by *Bacillus polymyxa* using lignocellulosic wastes. Ind. Crop. Products **7**, 195-203.
17. Pinaga, F., J. L. Pena and S. Valles. 1993. Xylanase production by *Bacillus polymyxa*. J. Chem. Tech. Biotechnol. **57**, 327-333.
18. Ratto, M., K. Poutanen and L. Viikari. 1992. Production of xylanolytic enzymes by an alkalitolerant *Bacillus circulans* strain. *Appl. Microbiol. Biotechnol.* **37**, 470-473.
19. Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. *Molecular Cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor,

U.S.A.

20. Srivastava, R. and A. K. Srivastava. 1993. Characterization of a bacterial xylanase resistant to repression by glucose and xylose. *Biotechnol. Lett.* **15**, 847-852.
21. Subramaniyan S. and P. Prema. 2002. Biotechnology of microbial xylanases: enzymology, molecular biology, and application. *Crit. Rev. Biotechnol.* **22**, 33-64.
22. Sunna, A., S. G. Prowe, T. Stoffregen and G. Antranikian. 1997. Characterization of the xylanases from the new isolated thermophilic xylan-degrading *Bacillus thermoleovorans* strain K-3d and *Bacillus flavothermus* strain LB3A. *FEMS Microbiol. Lett.* **148**, 209-216.
23. Viikari, L., A. Kantelinen, J. Sundquist and M. Linko. 1994. Xylanases in bleaching: from an idea to the industry. *FEMS Microbiol. Rev.* **13**, 335-350.
24. Wong, K. K. Y., L. U. L. Tan and J. N. Saddler. 1988. Multiplicity of β-1,4-xylanase in microorganisms: function and applications. *Microbiol. Rev.* **52**, 305-317.
25. Zamost, B. L., H. K. Nielson and R. L. Starnes. 1991. Thermostable enzymes for industrial applications. *J. Ind. Microbiol.* **8**, 71-82.

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초록 : *Paenibacillus* sp. DG-22로 부터 xylanase 생산의 최적화

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목재 저장소의 토양에서 분리된 호열성 세균인 *Paenibacillus* sp. DG-22로 부터 xylanase를 생산하기 위한 배양조건을 최적화시키기 위해 연구를 수행하였다. Xylanase생산은 세포의 생장과 연관된 양상을 나타내었다. Xylanase 활성은 배양상청액에서만 발견된 반면 β-xylosidase활성은 주로 세포와 결합되어 있었다. Xylanase 활성의 형성은 자일란에 의해 유도되었고 포도당과 자일로스에 의해서 억제되었다. 여러 상업적 자일란을 이용하여 xylanase의 생산양상을 조사한 결과 0.1-0.5%의 birchwood xylan에서 가장 높은 생산 율을 나타내었다. 조사된 여러 질소 원들 중 효모추출물이 xylanase 생산을 위하여 최적이었다. xylanase의 활성은 Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}와 Mn^{2+} 이온들에 의하여 억제된 반면 Ca^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+} 이온들과 DTT에 의해서는 촉진되었다. 수은은 5 mM의 농도에서 xylanase 활성을 완전히 파괴하였다. 자일란 가수분해의 주된 산물은 자일로바이오스, 자일로트라이오스 그리고 자일로 올리고당이었고 이것은 이 효소가 endoxylanase라는 것을 나타낸다.