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Research Article

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Cloning of new xylanase gene from *Bacillus brevis* in to *Escherichia coli*

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ABSTRACT

A gene for xylanase was isolated from Bacillus brevis and cloned in Escherichia coli BL21 host for heterologous gene expression. The xylanase gene was amplified by specifically designed primers in pUC vector while the PCR product was cloned in pET29a vector. Sequencing of the gene (GenBank accession number: bankit1383328 HQ179986) showed that it belongs to family 11 xylanases. The temperature and pH optima of B. brevis xylanase were found to be 55°C and 7.0, respectively. This xylanase gene was successfully expressed in BL21 with 30 IU/ml activity (~ 2 times higher than native host).

Keywords: *β-1, 4 Endo-xylanase, Bacillus brevis, Cloning, Vector*

INTRODUCTION

Xylan is a major component of hemicellulose of forest and agricultural materials such as hardwood, grain straw, corn cobs and grasses[29]. Xylan can be enzymatically degraded to xylose which can be further converted in to useful products like xylitol and ethanol[12,23,28]. Xylan is generally insoluble in nature; however, a number of microorganisms with the help of some of their enzymes can readily solubilize xylan. D-xylanase is one of the key enzymes required for the degradation of xylan. Molecular weight of xylanases varies from 15 to 30 kD, although higher molecular-weight xylanases have also been reported[7]. Studies on the cloning and molecular characterization of bacterial xylanases[8,9,10,13,16,17,22,25,26] suggest that this class of enzymes constitute a useful model for studies on protein secretion, expression, cloning, heterologous expression and for applied research. One of the exciting prospects for recombinant xylanase is its use in the paper and pulp industry to reduce the requirement of organo-chemicals for bleaching process. But, in this process, xylanase pretreatment has to take place at a high temperature and in alkaline conditions; due to this reason, thermostable xylanases with high pH optimum are of great importance. Therefore, an attempt was made to increase the xylanase production by heterologous gene expression so that further research could be done for its commercial applications. This paper reports the cloning, sequencing and heterologous expression of *Bacillus brevis* xylanase gene in *Escherichia coli* and also the analysis of some of its biochemical characteristics.

MATERIALS AND METHODS

Bacterial strains and culture media

Bacillus brevis strains, provided as cell pellets by Birla Institute of Science and Research (BISR), Jaipur, India and *E. coli* strain BL21, obtained from National Research Center on Plant Biotechnology (NRCPB), IARI, New Delhi, were used in the present study. LB medium was used for culture growth while LB agar was used for the maintenance of culture. M-9 supplemented with 1% xylan was used for xylanase production by *B. brevis*.

Isolation of bacterial genomic DNA and plasmid DNA

Total genomic DNA of the bacteria was isolated following the procedure of Ausubel *et al*[1]. with slight modifications. Cells were grown and harvested by centrifugation at 5000 x g for 10 min at 4ºC and pellet was re-suspended in 0.5 ml of TE buffer (10 mM Tris-Cl, pH 8-0, 1 mM EDTA), 1.5 ml of 10% (w/v) SDS and 50 µl of proteinase K (20 mg/ml) solution. The mixture was incubated at 37ºC for 1 hour. Following this, 1.8 ml of 5 M NaCl was added to the suspension and mixed thoroughly. The cells were lysed by adding 1.5 ml CTAB (Hexadecyl trimethyl ammonium bromide) / NaCl solution [10% (w/v) CTAB, 0.7 M NaCl] and incubated at 65ºC for 20 min. An equal volume of chloroform-isoamyl alcohol (24:1) was added to the mixture and DNA was extracted by inverting the tubes several times. This mixture was centrifuged at 6000 x g for 10 min at room temperature. The upper phase containing DNA was collected in a new tube and precipitated by adding 0.6 volumes of isopropanol. The spool of DNA was collected with sterile pipette tip in a new Eppendorf tube and washed with 70% ethanol, air dried and re-suspended in 0.5 ml TE buffer. Further purification of DNA was done by treating the sample with 5 µl RNAse (10 mg/ml) at 37ºC for 30 min followed by extraction with equal volume of phenol-chloroform-isoamyl alcohol (25:24:1). After centrifugation at 6000 x g for 5 min, the upper phase was collected in a new tube and DNA was precipitated by adding $1/10^{th}$ volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of absolute ethanol. The precipitate was incubated at -20ºC for 30 min and DNA was pelleted by centrifugation at 12000 x g for 10 min. The DNA pellet was washed with 70% ethanol, air dried and dissolved in 200 µl of TE buffer. Plasmid DNA was isolated using QIGEN plasmid purification kit.

PCR amplification of a partial fragment of the β-1, 4 endo-xylanase gene

For cloning of the β-1, 4 endo-xylanase gene of *B. brevis*, PCR primers were designed based on the sequence of the bacterial β-1, 4-endo-xylanase genes. The following primers were used for PCR amplification with the genomic DNA of *B. brevis* as a template.

5’CGGGGTACCTAAATGTTTAAGTTTAAAAAGAATTTCTTAGTTGG (forward primer)
5’ CGGAATTCTTACCACACTGTTACGTTAGAACTTCC (reverse primer).

The PCR was conducted in a GeneAmp PCR System 9700 (PE Biosystems) for 35 cycles (each cycle consisting of 1 min of denaturation at 93ºC, 1 min annealing at 55ºC, 1 min extension at 72ºC, and a final 10 min extension at 72ºC) using Taq Polymerase (Fermentas). Amplified PCR products were extracted from agarose gels by QIGEN DNA extraction kit and cloned in pUC vector.

Sub-cloning of primary clone

Plasmid DNA was first digested with appropriate enzyme(s), and the resulting fragments were separated on a 1.2% low-melting-point agarose gel. The sub-fragment to be re-cloned was isolated by QIGEN DNA extraction kit following the instructions of the supplier. Ligation of the purified sub-fragment with an appropriate vector and transformation of *E. coli* BL 21 was carried out.

Sequence analysis

Shotgun method was followed for sequencing the ‘Gene’ by using automatic sequencer (Applied Biosystems). A total of 6 clones were used for gene sequencing using universal primer present in pUC 19a. The sequencing was carried out at the Department of Biochemistry, University of Delhi South Campus, New Delhi. The search for amino acid sequences was performed with BLAST[2] or PSI-BLAST[3] while CLUSTAL W was used for the alignment of amino acid sequences.

Enzyme assays

The xylanase activity was determined with 1% birchwood xylan in 50 mM phosphate buffer (pH 7) at 55ºC using the method described by Bailey *et al*[4]. The enzymatic reaction was carried out for 5 min and the reducing sugars were determined using the DNS method[15]. The xylanase activity was measured in terms of international units (IU). One IU of xylanase is defined as one µ mole of xylose produced by 1 ml undiluted enzyme in 1 min. The µ moles of xylose produced by xylanase were deduced from xylose standard plot.

SDS-PAGE and zymogram

Polyacrylamide (12.5%) gel electrophoresis was performed following standard methods as described by Laemmli[14] (1970). Proteins were visualized by incubating gels with gentle shaking for 30 min in 10% trichloroacetic acid, 4 h in Coomassie blue staining solution {45% (v/v) ethanol, 10% (v/v) acetic acid, 0.25% (w/v) Coomassie brilliant blue R250}, and overnight in de-staining solution {67% (v/v) water , 25% (v/v) ethanol, 8% (v/v) acetic acid}.

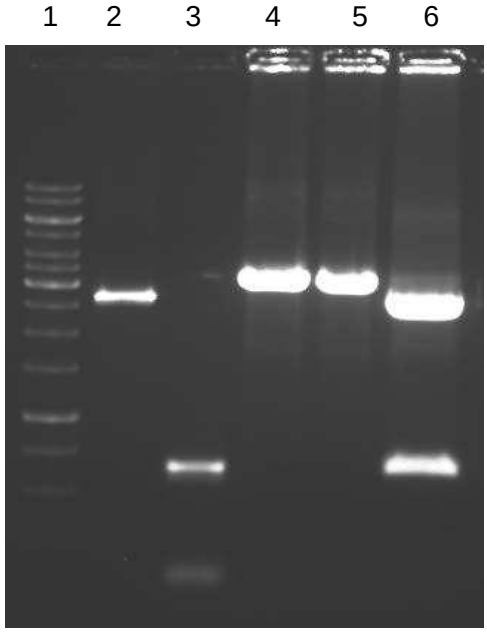
For the zymogram analysis, the crude enzyme samples were electrophoresed as above on SDS-PAGE containing xylan (0.1%). After running, the gel was washed four times for 30 min in 100 mM phosphate buffer (pH 7.0); the first two washes containing 25% (v/v) isopropyl alcohol, to remove SDS and renature protein in the gel. The gel was then incubated for 20 min at 37**ºC** before soaking in Congo red solution for 5 min at room temperature and washing with 1 M NaCl until excess dye was removed from the active band. The zymogram was prepared after soaking the gel in 0.5% acetic acid solution. The background turned dark blue, and clear zones were observed in the areas exposed to xylanase activity[21].

RESULTS

Cloning of *B. brevis* xylanase gene in pUC vector

PCR amplified gene was cloned in *E. coli* DH5α by pUC19 vector and transformed cells were screened by blue/white colony selection. Initially, 8 colonies were identified and screened by restriction digestion (*Kpn*I and *Eco*RI) analysis. Out of these 8 colonies, one colony was selected for further studies. A fragment of ~700 bp (Fig. 1) was inserted out on digestion with *Kpn*I and *Eco*RI endonucleases. This fragment has the same size as that of the PCR amplified product.

Fig. 1. pUC cloning

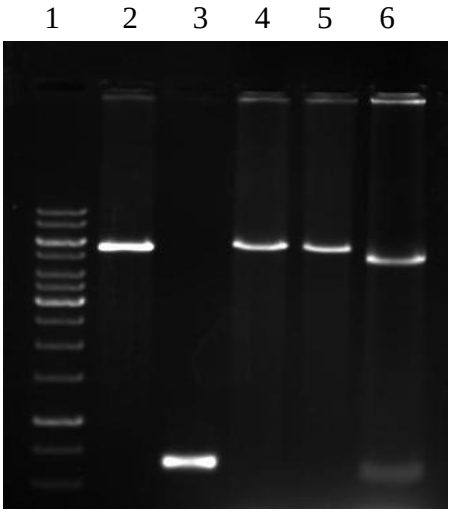


Lane 1: 1 kb DNA marker, Lane 2: plasmid (pUC) linearized, Lane 3: xylanase gene (PCR product), Lane 4: clone linearized (*Kpn*I digested), Lane 5: clone linearized (*Eco*RI digested), Lane 6: clone double digest (*Kpn*I and *Eco*RI), gene insert out.

Sub-cloning of *B. brevis* xylanase in pET29a

The *Kpn*I-*Eco*RI double digest insert was taken out from pUC vector and used for ligation of xylanase gene in pET29. After transformations of DH5α, a total of 6 colonies were selected and screened; of which, one was selected for further studies. The positive clone inserted out a single fragment of desired size (Fig. 2). For expression of the xylanase gene, the expression host BL21 was transformed by the isolated plasmid of + ve clone.

Fig. 2: pET cloning

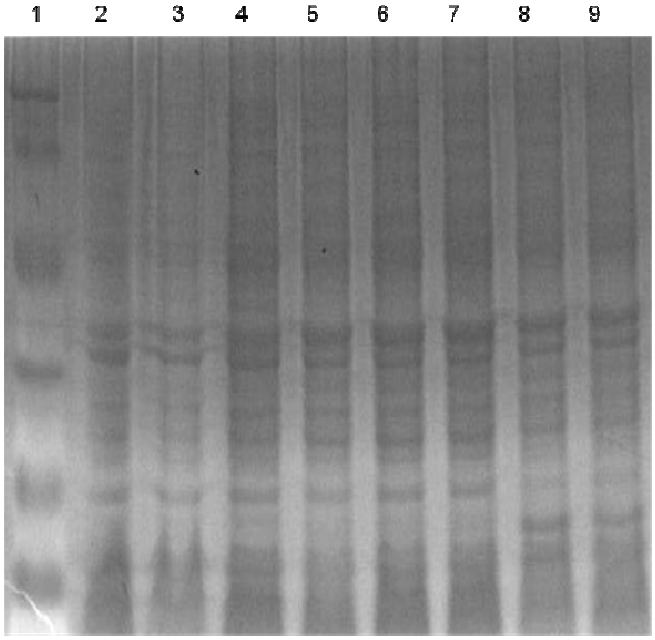


Lane 1: 1 kb DNA marker, Lane 2: plasmid linearized, Lane 3: xylanase gene (PCR product), Lane 4: clone linearized by single digest (*Kpn*I), Lane 5: clone linearized single digest (*Kpn*I), and Lane 6: clone double digest (*Kpn*I and *Eco*RI), gene insert out.

SDS-PAGE and zymogram

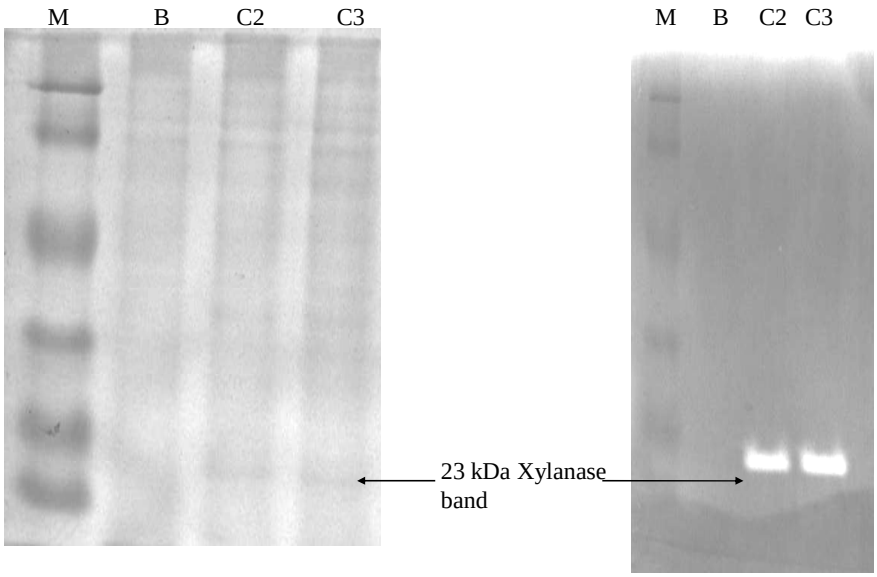
SDS-PAGE was performed using two expression clones (clones 2 and 3) with and with-out IPTG induction (Fig. 3). The zymogram (showing xylanase activity on the gel) was developed (Fig. 4) and showed xylanase activity by clearing zone development on staining with Congo red dye. The molecular weight of *B. brevis* xylanase was found to be 23 kDa.

Fig. 3: SDS-PAGE of clones for xylanase gene expression



Lane 1: Marker, Lane 2: Clone 2 with-out induction, Lane 3: Clone 3 with-out induction, Lane 4: IPTG induced control (BL21) cell protein, Lane 5: IPTG induced clone 2 cell protein, Lane 6: IPTG induced clone 3 cell protein, Lane 7: Control (BL21) broth, Lane 8: IPTG induced clone 2 broth, and Lane 9: IPTG induced clone 3 broth.

Fig. 4: SDS-PAGE and zymogram of expressed xylanase



Xylanase production before and after cloning

Xylanase production was found more (approximately 1.5 times higher) in cloned expression host as compared to *B. brevis*. The optimization of temperature and pH of both cloned and native (*B. brevis*) xylanases are shown in Figures 5 and 6, respectively.

Fig.5: Incubation temperature optimization for xylanase activity

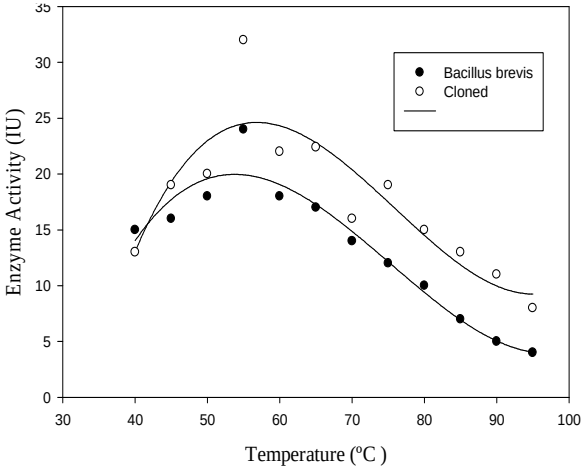
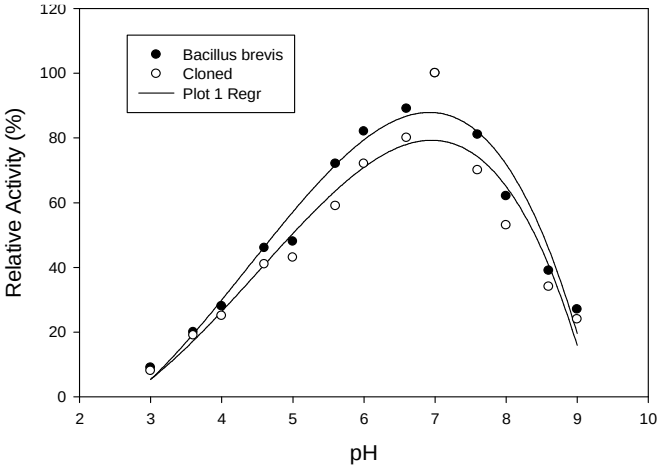


Fig.6: pH optimization of xylanase activity



Sequence analysis

The sequence of *B. brevis* xylanase was submitted to Gen Bank (**accession number: bankit1383328 HQ179986**). BLAST (http://www.ncbi.nlm.nih.gov) was used for similarity search to explore all of the available sequence. The scores assigned in a BLAST search have a well-defined statistical interpretation, making real matches easier to distinguish from random background hits.

Contig Assembly: CAP3 was used for finding the contig from forward and reverse sequencing results. The contig of all the gene sequence are as follows:

>**Contig of clone 3**

TGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATT
AAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCC
AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCTAAATGTTTAAGT
TTAAAAAGAATTTCTTAGTTGGATTAACGGCAGCTTTAATGAGTATTAGCTTGTTTTCG
GCAACCGCCTCTGCAGCTAGCACAGACTACTGGCAAAATTGGACTGATGGGGGCGGA
ATAGTAAACGCTGTCAATGGGTCTGGCGGGAATTACAGTGTTAATTGGTCTAATACCG
GAAATTTCGTTGTTGGTAAAGGTTGGACTACAGGTTCGCCATTTAGGACAATAAACTA
TAATGCCGGAGTTTGGGCGCCGAATGGCAATGGATATTTGACTTTATATGGTTGGACG
AGATCACCTCTCATAGAATATTATGTAGTGGATTCATGGGGTACTTATAGACCAACAG
GAACGTATAAAGGTACTGTAAACAGTGATGGGGGTACATATGACATATATACAACTAC
ACGTTATAACGCACCTTCCATTGATGGCCCAAGCACTACTTTTACGCAGTACTGGAGT
GTTCGTCAGTCGAAGAGACCAACTGGAAGCAACGCTACAATCACTTTCAGCAATCATG
TTAACGCATGGAAGAGTCATGGAATGAATCTGGGTAGCAATTGGTCTTACCAAGTCTT
AGCGACAGAGGGATATCAAAGTAGTGGAAGTTCTAACGTAACAGTGTGGTAAGAATT
CGTAATCATGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACA
ACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAAT

DISCUSSION

Many xylanase genes have been isolated from different microbial organisms and were expressed in *E. coli*. However, the level of xylanase expression in *E. coli* was generally lower than the parent organism, and confined mainly to the cytoplasmic or periplasmic fractions. Hyper-expression of a *B. circulans* xylanase gene in *E. coli* was reported by Yang *et al*[30]. with a xylanase activity of 7 IU/ml found in intracellular fraction of *E. coli*. Extra-cellular activity of xylanase had also been reported earlier in recombinant *E. coli* for the bacterial xylanases from alkaliphilic *Bacillus* by Honda[11], and alkaliphilic as well as thermophilic *Bacillus* species by Shendye and Rao[24]. Most of the reports on heterologous xylanase gene expression have shown low xylanase activity in *E. coli* over that of the original organism[5,19,20]. Expression of *B. subtilis* xylanase gene (modified to *B. circulans* xylanase gene via single codon substitution) in *E. coli* was reported by Sung *et al*[27]. A synthetic gene encoding mature *B. circulans* xylanase has been designed to imitate the frequency of degenerate codons used in *E. coli*. This synthetic gene was then converted to *B. subtilis* xylanase gene via substitution of Thr^{147} Ser codon. Under the control of *lac* promoter, recombinant xylanase activity in solution form in cytoplasm was reported as high as 60 IU/ml.

In the present study, we have isolated a gene for xylanase from *B. brevis* and cloned the same in *E. coli* following two step cloning process. In the first step, xylanase gene was cloned in DH5α by pUC19 and in the second step, for expression of xylanase gene, cloning was done in BL21 by pET29a. We understand that this is the first report on cloning and heterologous expression of *B. brevis* xylanase gene in BL21 host. The most significant part of this study is the expression of cloned xylanase gene in extracellular fluid of *E. coli* which may be exploited for production of xylanase at commercial level. It is interesting that the xylanase activity in the *E. coli* clone is higher (1.5 times) over that of native *B. brevis* strain. The reason for this high activity is not clear. The enzyme produced by *E. coli* is functionally active and capable of degrading birchwood xylan even in SDS-PAGE gel also. However, the structure and characteristics of this enzyme in *B. brevis* and *E. coli* have yet to be determined. Bacterial xylanases tend to be monomers having molecular weights in the range of 24 kDa to 145 kDa[18].

Further studies on the DNA sequence of the cloned fragment containing xylanase gene should prove helpful in determining the regulatory sequences of the gene and the subunit structure of this enzyme.

CONCLUSION

B. brevis xylanase was efficiently expressed and secreted by *E. coli* (BL21), the xylanase activity was found more (1.5 times) in culture filtrate of BL21 as compared to *B. brevis*. Heterologous expression systems that produce large amounts of secreted proteins within the organism that can be grown in industrial scale fermenters must be developed to facilitate higher enzyme production by using agri-waste as a carbon source for enzyme production. Since optimization of growth medium is a useful tool to attain high levels of enzyme activity at lower cost[6], further studies on medium optimization might improve the yield of *B. brevis* xylanase production with the kind of BL21 expression systems described in this paper. An important further consideration is to develop higher-level enzyme producing integrant *E. coli* strains with several copies of *B. brevis* xylanase for growth in optimized media. BLAST results (data not shown) reveal that this sequence is a new sequence and has not been reported or submitted to gene bank by any group/researcher till now. So we report the sequence of a new gene of xylanase.

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Conflict of Interest

The Authors declare that they have no conflict of interest

REFERENCES

1. Ausubel, F.M. Brent, R. Kingston, R.E. Moore, D.D. Seidman, J.G. Smith, J.A. & Struhl, K., Short Protocols in Molecular Biology, John Wiley & Sons, New York, Ch. 2.4 (1995)
2. Altschul, S.F. Gish, W. Miller, W. Myers, E.W. & Lipman, D.J., Basic local alignment search tool. *J Mol Biol.*, **215:** 403–410 (1990)
3. Altschul, S.F. Madden, T.L. Schaffer, AA. Zhang, J. Zhang, Z. Miller, W. & Lipman, D.J., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, **25:** 3389–3402 (1997)
4. Bailey, M.J. Biely, P. & Poutanen, K., Interlaboratory testing of methods for assay of xylanase activity. *J Biotechnol.*, **23:** 257-270 (1992)
5. Bernier, R. Driguez, Jr. H. & Desrochers, M., Molecular cloning of a *Bacillus subtilis* xylanase gene in *Escherichia coli*. *Gene.*, **26:** 59-65 (1983)
6. Damaso, M.C.T. Almeida, M.S. Kurtenbach, E. Martins, O.B. Pereira, N. Andrade, Jr. C.M.M.C. & Albano, R.M., Optimized expression of a thermostable xylanase from *Thermomyces lanuginosus* in *Pichia pastoris. Appl Environ Microbiol.,* **69:** 6064-6072 (2003)
7. Dekker, R.F.H. & Richards, G.N., Hemicelluloses: their occurrence, purification, properties, and mode of action. *Adv Carbohydr Chem Biochem* **32:** 276-352 (1976)
8. Fukusaki, E. Panbangered, W. Shinmyo, A. & Okada, H., The complete nucleotide sequence of the xylanase gene (*xynA*) of *Bacillus pumilus*. *FEBS Lett.,* **171:** 197-201 (1984)
9. Guo, B. Li, P.Y. Yue, Y.S. Zhao, H.L. Dong, S. Song, X.Y. Sun, C.Y. Zhang, W.X. Chen, X.L. Zhang, X.Y. Zhou, B.C. & Zhang, Y.Z., "Gene Cloning, Expression and Characterization of a Novel Xylanase from the Marine Bacterium, *Glaciecola mesophila* KMM241" *Mar. Drugs.*, **11:** 1173-1187; doi:10.3390/md11041173 (2013)
10. Hamamoto, T. Honda, H. Kudo, T. & Horikoshi, K., Nucleotide sequence of the xylanase A gene of alkalophilic *Bacillus* sp. strain C-125. *Agric Biol Chem.,* **51:** 953-955 (1987)
11. Honda, H. Kudo, T. & Horikoshi, K., Molecular cloning and expression of the xylanase gene from alkalophilic *Bacillus* strain C-125 in *Escherichia coli. J Bacteriol* **161:** 784-785 (1985)

12. Jeifries, T.W., Conversion of xylose to ethanol under aerobic conditions by *Candida tropicalis*. *Biotechnol Lett* **3:** 213-218 (1981)
13. Je-Ruei, L. Duan, C.H. Zhao, X. Jason, T.C. Tzen. Cheng, T.K.J. & Pai, C.K., Cloning of a rumen fungal xylanase gene and purification of the recombinant enzyme via artificial oil bodies. *Appl Microbiol Biotechnol.,* **79:** 225–233 (2008)
14. Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage-T4. *Nature* **227:** 680-685 (1970)
15. Miller, G.L., The use of dinitrosalicylic acid reagent for the determination of reducing sugars. *Anal Chem.,* **31**: 426-428 (1959)
16. Moriyama, H. Hata, Y. Yamaguchi, H. Sato, M. Shinmyo, A. Tanaka, N. Okada, H. & Katsube, Y., Crystallization and preliminary X-ray studies of *Bacillus pumilus* IPO xylanase. *J Mol Biol* **193:** 237-238 (1987)
17. Paice, M. Bourbonnais, R. Desrochers, M. Jurasek, L. & Yaguchi, M., A xylanase gene from *Bacillus subtilis*: nucleotide sequence and comparison with *B. pumilus* gene. *Arch Microbiol.,* **144:** 201-206 (1986)
18. Panbangered, W. Shinmyo, A. Kinoshita, S. & Okada, H., Purification and properties of endoxylanase produced by *Bacillus pumilus. Agric Biol Chem.,* **47:** 957-963 (1983)
19. Panbangered, W. Kawaguchi, O. Tomita, T. Shinmyo, A. & Okada, H., Isolation of two B-xylosidase genes of *Bacillus pumilus* and comparisions of their gene products. *Eur J Biochem* **138:** 267-273 (1984)
20. Panbangered, W. Fukusake, E. Epifanio, E. Shinmyo, A. & Okada, H., Expression of a xylanase gene of *Bacillus pumilus* in *Escherichia coli* and *Bacillus subtilis*. *Appl Microbiol Biotechnol.,* **22:** 259-264 (1985)
21. Rawashdeh, R. Saadoun, I. & Mahasneh, A., Effect of cultural conditions on xylanase production by *Streptomyces sp.* (strain Ib 24D) and its potential to utilize tomato pomace. *African J Biotechnol.,* **4(3):** 251-255 (2005)
22. Sandhu, J.S. & Kennedy, J.F., Molecular cloning of *Bacillus polymyxa* (1- 4)-P-D-xylanase gene in *Escherichia coli*. *Enzyme Microb Technol.,* **6:** 271-274 (1984)
23. Schneider, H. Wang, P.Y. Chan, Y.K. & Maleszka, R., Conversion of D-xylose into ethanol by the yeast *Pachysolen tannophilus. Biotechnol Lett.,* **3:** 89-92 (1981)
24. Shendye, A. & Rao, M., Cloning and extracellular expression in *Escherichia coli* of xylanase from an alkalophilic thermophilic *Bacillus* sp. NCIM 59. *FEMS Microbiol Lett.,* **108:** 297-302 (1993)
25. Shi, P. Du, Y. Yang, H. Huang, H. Zhang, X. Wang, Y. & Xao, B., Molecular Characterization of a new Alkaline-Tolerent Xylanase form *Humicola insolens* Y1. BioMed Res. Int., **2015:** (2015)
26. Sipat, A. Taylor, K.A. Lo, R.Y.C. Forsberg, C.W. & Kreli, P.J., Molecular cloning of a xylanase gene from *Bacteroides succinogenes* and its expression in *Escherichia coli*. *Appl Environ Microbiol.,* **53:** 477-481 (1987)
27. Sung, W.L. Luk, C.K. & Zahab, D.M., Over expression and purification of the *Bacillus subtilis* and *Bacillus circulans* xylanases in *Escherichia coli*. *Protein Express Purifi.,* **4:** 200-206 (1993)
28. Wang, P.Y. Johnson, B.F. & Schneider, H., Fermentation of D-xylose by yeasts using glucose isomerase in the medium to convert D-xylose to D-xylulose. *Biotechnol Lett.,* **2:** 273-278 (1980)
29. Wilkie, K.C.B., The hemicelluloses of grasses and cereals. *Adv Carbohyr Chem Biochem.,* **36:** 215-262 (1979)
30. Yang, R.C.A. Mackenzie, R.C. Bilous, D. & Narang, S.A., Hyperexpression of a *Bacillus circulans* xylanase gene in *Escherichia coli* and characterization of the gene product. *Appl Environ Microbiol.,* **55:** 1192-1195 (1989)