CLONING OF XYLANASE GENE FROM *Trichoderma reesei* ATCC 58350

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abstract

Gene encoding xylanase from *Trichoderma reesei* ATCC 58350 was amplified using Polymerase Chain Reaction. Both genomic and cDNA of the xylanase gene were obtained and analysed. Comparison of the nucleotide sequence shows the existence of one intron. Amino acid sequence comparison with other xylanase exhibited 100% identities to xylanase from *Hypocrea jecorina* QM6a.

introduction

The main portion of hemicelluloses is xylan which is a polymer consisting primarily of beta-1,4 bonds residues are digested by xylanase enzymes (Prade, 1996). Apart from that, there are assortments of other enzymes that remove various chemical side chains that are attached to the main xylan in concert to synergistically hydrolyze xylan.

The xylanase enzymes can be used in conjunction with cellulose enzymes to hydrolyze the lignocellulose substrate into sugars that can then be fermented by microorganisms into products such as ethanol and other value-added fermentation products. Recently, there has been an increasing interest in applying xylanases to pulping processes. Other than that, xylanases increase the digestibility of feed by lowering the viscosity in the intestinal tract, thus improving nutrient uptake (Twomey *et* al., 2003). Applications have also been found in textile manufacture, in baking, and also in beer and juice processing.

materials and methods

Growth of fungal strain: *T.reesei* was induced with oat spelt

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Extraction of genomic DNA and mRNA: PCR and RT-PCR were carried out to amplify xylanase gene

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Clone into cloning vector: The gene of interest was then cloned into pTZ57R/T vector.

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DNA sequencing: Amino acid and nucleotide analysis

results and discussion

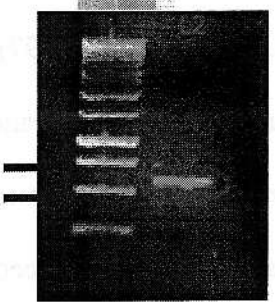


Figure 1 : Amplified fragment of *T.reesei xyn I* cDNA *(573bp)*

Lane 1 : 1kb DNA marker
Lane 2: cDNA (573bp)

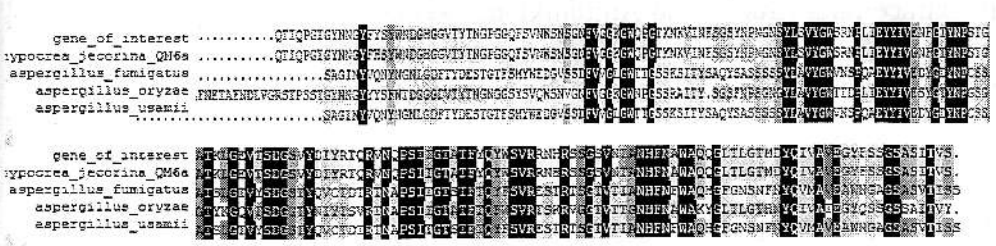


Figure 2: Amino acids sequence comparison of xylanase from various fungi. The gene encoded 221 amino acid sequence of xylanase. Amino acid analysis shows 100% identities to xylanase from *Hypocrea jecorina* QM6a

References

Bajpai, P. Microbial xylanolyic enzyme system: properties and applications. Advance Applied Microbiology, 43:141-194, 1997.

Biely, P., MacKenzie, C.R., Puls, J. and Schneider, H. Cooperativity of esterases and xylanases in the enzymic degradation of acetyl xylan. Biotechnology 4: 731-733, 1986.

Prade, R.A. Xylanases: from biology to biotechnology. Biotechnology Genetic Engineering Review 13:101-131, 1996.

Twomey L.N., Pluske J.R., Rowe J.B., Choct M., Brown W., McConnell M.F., Pethick D.W. The effects of increasing levels of soluble non-starch polysaccharides and inclusion of feed enzymes in dog diets on faecal quality and digestibility. Animal Feed Science and Technology: 108(1-4): 71-82, 2003.

Title: Cloning of Xylanase from *Trichoderma reesei* ATCC 58350

1.1 Introduction

Plant cell walls are comprised of cellulose and hemicellulose and other polymers that are intertwined together to form a complex structure. Hemicelluloses are the second most abundant polysaccharides in nature. It composed of heterogeneous polymers such as pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids. The main portion of hemicellulose is xylan which is a polymer consisting primarily of beta-1,4-linked xylose residues.

In nature, the beta-1,4 bonds between the xylose residues are digested by xylanase enzymes (Prade, 1996). The endo-xylanases digest the internal β-1,4 bonds. Apart from that, there are an assortment of other enzymes that remove various chemical side chains that are attached to the main xylan polymer (Biely *et* al., 1986, Bajpai, 1997). These enzymes are β-xylosidase, and several accessory enzymes such as α-arabinofuranosidase, α-glucuronidase, and acetylxylan esterase. All of these enzymes work in concert to synergistically hydrolyze xylan. In addition, these enzymes are produced by a number of bacteria and fungi and are mostly extracellular (Sunna and Antranikian, 1997).

Among microbial sources, the filamentous fungi are well known as secretor of high level of xylanase enzymes into the culture medium. This property makes fungi

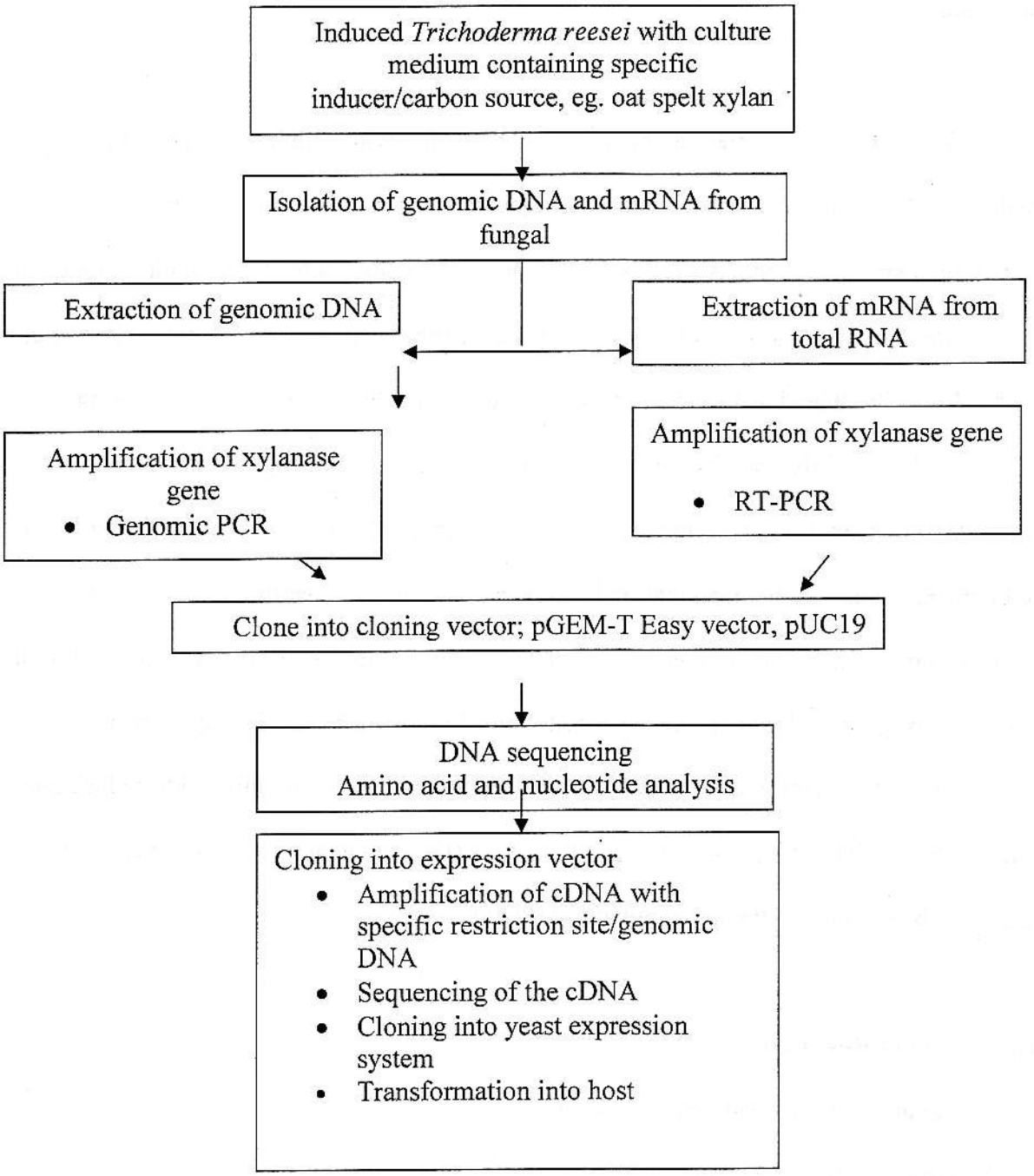
economically effective producers of xylanases, which are widely used in various industrial applications.

The xylanase enzymes can be used in conjunction with cellulase enzymes to hydrolyze the lignocellulose substrate into sugars that can then be fermented by microorganisms into products such as ethanol and other value-added fermentation products. Apart from that, it can also be used alone to produce purer cellulose preparations (Beg *et* al., 2001). Recently, there has been an increasing interest in applying xylanases to pulping processes. Particularly, they have been used to facilitate the bleaching of kraft pulps or to improve fiber properties. Thus decreasing the chemical usage and costs normally associated with this procedure. Other than that, xylanases increase the digestibility of feed by lowering the viscosity in the intestinal tract, thus improving nutrient uptake (Twomey *et* al., 2003). In baking, they are added to increase the specific bread volume and in this way improve final flavor (Maat *et* al., 1992). In beer and juice processing, xylanases are utilized to reduce haze formation by solubilizing long chain arabinoxylans (Dervilly *et* al., 2002). Applications have also been found in textile manufacture (Prade, 1995).

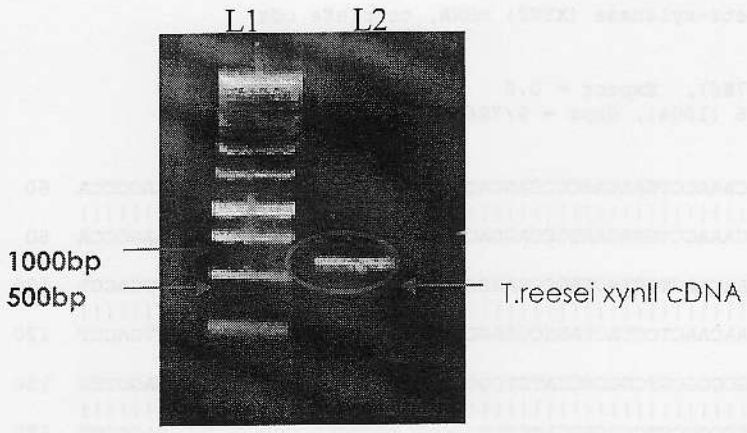
1.2 Scopes of Research

1. Isolation mRNA and genomic DNA.
2. Amplification of xylanase cDNA using RT-PCR.
3. Cloning and expression of xylanase gene in yeast system.
4. Biochemical characterization of expressed xylanase.
5. Optimization and production of the recombinant xylanase.

1.3 Materials and Methods



RESULTS AND DISCUSSION



Amplified fragment of *T.reesei* xynII cDNA (~573bp). Agarose gel electrophoresis of *Trichoderma reesei* xynII cDNA.

Lane 1: 1kb DNA marker

Lane 2: *Trichoderma reesei* cDNA showing band with expected size (~573bp)

- Sequence of cloned xylanase gene without signal peptide from *Trichoderma reesei.*

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Trichoderma reesei beta-xylanase (XYN2) mRNA, complete cds
Length=786

 Score = 1452 bits (786),  Expect = 0.0
 Identities = 786/786 (100%), Gaps = 0/786 (0%)
 Strand=Plus/Plus

Query  1    GAATTCGCCAAACCTGAACAACCCCAGCACCTGAACAGTCATACAACCCCTCCAAGCCCA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GAATTCGCCAAACCTGAACAACCCCAGCACCTGAACAGTCATACAACCCCTCCAAGCCCA  60

Query  61   AAAGACACAACAACTCCTACTAGCCGAAGCAAGAAGACATCAACATGGTCTCCTTCACCT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AAAGACACAACAACTCCTACTAGCCGAAGCAAGAAGACATCAACATGGTCTCCTTCACCT  120

Query  121  CCCTCCTCGCCGGCGTCGCCGCCATCTCGGGCGTCTTGGCCGCTCCCGCCGCCGAGGTCG  180
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Sbjct  121  CCCTCCTCGCCGGCGTCGCCGCCATCTCGGGCGTCTTGGCCGCTCCCGCCGCCGAGGTCG  180

Query  181  AACCCGTGGCTGTGGAGAAGCGCCAGACGATTCAGCCCGGCACGGGCTACAACAACGGCT  240
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1.4 References

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2. Biely, P., MacKenzie, C.R., Puls, J. and Schneider, H. Cooperativity of esterases and xylanases in the enzymic degradation of acetyl xylan. Biotechnology 4: 731-733, 1986.
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4. Twomey L.N., Pluske J.R., Rowe J.B., Choct M., Brown W., McConnell M.F., Pethick D.W. The effects of increasing levels of soluble non-starch polysaccharides and inclusion of feed enzymes in dog diets on faecal quality and digestibility. Animal Feed Science and Technology: 108(1-4): 71-82, 2003.