

Enhancement of the
Trichoderma reesei
expression system

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Table of Contents

TABLE OF CONTENTS	I
LIST OF FIGURES	V
LIST OF TABLES	VII
ABBREVIATIONS	VII
DECLARATION	X
ACKNOWLEDGEMENTS	XI
ABSTRACT	VIII
CHAPTER 1: INTRODUCTION	1
1.1 WORLD DEMAND FOR ENZYMES	1
1.2 FILAMENTOUS FUNGI AND ENZYME PRODUCTION	1
1.3 TRICHODERMA REESEI	3
1.4 ENZYMES OF TRICHODERMA REESEI FOR BIOMASS DEGRADATION	4
1.4.1 <i>Domain conformations of cellulolytic and xylanolytic enzymes</i>	8
1.5 PROTEIN EXPRESSION SYSTEMS	10
1.5.1 <i>Production of xylanases by Trichoderma reesei</i>	17
1.6 ENHANCEMENT OF HETEROLOGOUS EXPRESSION IN FILAMENTOUS FUNGI	22
1.6.1 <i>Expression under strong fungal promoters</i>	22
1.6.2 <i>Introduction of exogenous genes into filamentous fungi</i>	30
1.6.3 <i>Gene fusion approach</i>	32
1.6.4 <i>Homologous and ectopic integration of transforming DNA</i>	35
1.6.5 <i>Gene copy number</i>	35
1.6.6 <i>Optimisation of codon usage of the incoming genes</i>	36
1.6.7 <i>Hypercellulolytic mutant strain T. reesei RUT-C30</i>	37
1.6.8 <i>Overview of the secretory pathway in filamentous fungi</i>	38
1.6.9 <i>Protein degradation by proteases</i>	40
1.6.10 <i>Protein glycosylation in filamentous fungi</i>	43
1.7 THE CONCEPT OF THE MULTIPLE PROMOTER PLATFORM (MPP)	47
1.8 AIMS OF THIS PROJECT	47
CHAPTER 2: MATERIALS AND METHODS	49
2.1 COMMON CHEMICALS AND SOLUTIONS	49
2.2 BACTERIAL AND FUNGAL STRAINS	49
2.2.1 <i>Escherichia coli strain</i>	49
2.2.2 <i>Trichoderma reesei strains</i>	49
2.3 MEDIA AND CULTURE CONDITIONS	50
2.3.1 <i>Bacterial growth media</i>	50
2.3.2 <i>Fungal growth media</i>	50
2.3.3 <i>Culture conditions</i>	51
2.3.4 <i>Selection of fungal transformants</i>	51
2.4 COMPUTATIONAL ANALYSIS	52
2.5 MOLECULAR CLONING PROCEDURES	53
2.5.1 <i>Extraction of fungal genomic DNA (gDNA)</i>	53
2.5.2 <i>Restriction endonuclease digestion</i>	53
2.5.3 <i>Ligations</i>	53
2.5.4 <i>Phosphatase treatment of DNA fragments</i>	53
2.5.5 <i>Polymerase chain reaction</i>	54
2.5.6 <i>Primers for PCR</i>	54
2.5.7 <i>DNA sequencing</i>	54

2.5.8 DNA hybridisation and Southern blotting.....	54
2.6 PROTEIN ANALYSIS	56
2.6.1 Protein quantification	56
2.6.2 Two dimensional gel electrophoresis of fungal culture supernatants	56
2.7 ANALYSIS OF XYLANASE B PRODUCED BY T. REESEI TRANSFORMANTS.....	57
2.7.1 Purification of recombinant Xylanase B by heat treatment	57
2.7.2 Detection of Xylanase B.....	57
2.8 FUNGAL TRANSFORMATION USING BIOLISTIC BOMBARDMENT	59
2.8.1 Preparation of DNA-coated gold microparticles	59
2.8.2 Preparation of conidia for transformation.....	60
2.8.3 Standard bombardment procedure	60
CHAPTER 3: IDENTIFICATION OF STRONG PROMOTERS FROM TRICHODERMA REESEI AND CONSTRUCTION OF GENE EXPRESSION CASSETTES	61
3.1 INTRODUCTION.....	61
3.2 MATERIALS AND METHODS	62
3.2.1 Stability of Xylanase B at different pH's.....	62
3.2.2 Two dimensional electrophoresis and protein identification.....	62
3.2.3 Design of gene expression vectors.....	63
3.3 RESULTS AND DISCUSSION.....	71
3.3.1 The effect of pH on the yield of Xylanase B	71
3.3.2 Identification of highly secreted proteins by 2-DE analyses.....	74
3.3.3 Five gene expression vectors.....	80
3.4 SUMMARY	83
CHAPTER 4: HETEROLOGOUS PROTEIN EXPRESSION BY SINGLE- PROMOTER APPROACH	84
4.1 INTRODUCTION.....	84
4.2 MATERIALS AND METHODS	86
4.2.1 Generation and analysis of transformants	86
4.2.2 Determination of gene copies and homologous integration of the gene expression cassettes.....	87
4.3 RESULTS AND DISCUSSION.....	87
4.3.1 Generation and screening of single-promoter strains.....	89
4.3.2 Efficiency of transformation with the five gene expression cassettes.....	96
4.3.3 Overall comparison of Xylanase B activity in all selected transformants using liquid enzyme assay	97
4.3.4 Selection of expression vectors for generation of strains with multiple expression vectors	101
4.3.5 Gene copy number and homologous integration of single-promoter strains...	101
4.3.6 Inconsistent digestion of gDNA fragments.....	106
4.3.7 Comparison of gene copy numbers and Xylanase B activity in CBH2sigpro, CBH2cbmlin, EGL2sigpro and EGL2cbmlin transformants	107
4.3.8 Comparison of gene copy numbers and xylanase B activity in XYN2sigpro transformants	109
4.3.9 Effects of homologous and non-homologous integration on Xylanase B activity in XYN2sigpro (XS) strains.....	110
4.4 SUMMARY AND NEXT STEPS	112
CHAPTER 5: HETEROLOGOUS PROTEIN EXPRESSION BY MULTIPLE- PROMOTER APPROACH	113
5.1 INTRODUCTION.....	113

5.2 MATERIALS AND METHODS	115
5.2.1 <i>Strains</i>	115
5.2.2 <i>Screening of potential transformants</i>	115
5.2.3 <i>DNA hybridisation for identification of the number of gene copies</i>	117
5.3 RESULTS AND DISCUSSION	119
5.3.1 <i>Generation of multiple-promoter transformants</i>	119
5.3.2 <i>Selection of the best multiple-promoter strain</i>	123
5.3.3 <i>Gene copy number and homologous integration of the gene cassettes in multiple-promoter strains</i>	129
5.3.4 <i>Comparison of the Xylanase B activity produced by the best single-promoter and multiple-promoter transformants</i>	138
5.4 SUMMARY	143
CHAPTER 6: GLYCOSYLATION OF XYLANASE B EXPRESSED BY SELECTED TRANSFORMANT STRAINS.....	144
6.1 INTRODUCTION	144
6.2 MATERIALS AND METHODS	145
6.2.1 <i>Overview of the procedure</i>	145
6.2.2 <i>Transformant strains</i>	145
6.2.3 <i>Quantification of Xylanase B by Densitometry</i>	146
6.2.4 <i>Digestion with N-glycosidase</i>	146
6.2.5 <i>Prediction of N- and O-linked glycosylation sites</i>	147
6.2.6 <i>Peptide mass calculation of the digested recombinant Xylanase B protein</i>	147
6.2.7 <i>Release of O- and N-linked glycans from Xylanase B</i>	147
6.2.8 <i>Analysis of N- and O-linked glycans by LC-ESI MS/MS</i>	147
6.3 RESULTS AND DISCUSSION	148
6.3.1 <i>Detection of Xylanase B from the best transformant strains</i>	148
6.3.3 <i>Prediction of potential N- and O-linked glycosylation sites on Xylanase B</i> ...	153
6.3.4 <i>Digestion of Xylanase B with N-Glycosidase F</i>	153
6.3.5 <i>Identification of N- and O-linked glycans</i>	155
6.3.6 <i>Fusion of the EGLII CBM and linker with Xylanase B</i>	158
6.3.7 <i>Glycosylated Xylanase B and the level of production</i>	159
6.3.8 <i>O-linked hexuronic acid in the recombinant Xylanase B</i>	160
6.4 SUMMARY	163
CHAPTER 7: SUMMARY, CONCLUSIONS AND FUTURE WORK	164
7.1 ENHANCEMENT OF THE TRICHODERMA REESEI EXPRESSION SYSTEM	164
7.1.1 <i>Identification of strong promoters from Trichoderma reesei and construction of gene expression cassettes</i>	166
7.1.2 <i>Heterologous protein expression by the single-promoter approach</i>	167
7.1.3 <i>Heterologous protein expression by the multiple-promoter approach</i>	167
7.1.4 <i>Glycosylation of Xylanase B expressed from the selected strains</i>	168
7.2 FUTURE WORK	168
7.2.1 <i>Transformation by biolistic bombardment with multiple DNA cassettes</i>	168
7.2.2 <i>One-by-one introduction of expression vectors</i>	169
7.2.3 <i>The number of promoters expressing Xylanase B</i>	170
7.2.4 <i>Enhancing production in a cbhI knockout strain</i>	170
7.2.5 <i>Location of gene cassettes in the best recombinants</i>	171
7.2.6 <i>Factors contributing to protein production</i>	172
7.2.7 <i>A rapid quantification method of Xylanase B is required</i>	173
7.2.8 <i>Specific activity of Xylanase B expressed by Trichoderma reesei</i>	173
7.2.9 <i>Further analysis of N- and O-linked glycans of the recombinant Xylanase B</i> ..	175
7.3 CONCLUSION	175

BIBLIOGRAPHY	177
APPENDICES	204
APPENDIX A.....	204
<i>DNA sequence of the gene expression cassettes</i>	<i>204</i>
APPENDIX B.....	207
<i>DNA and protein sequences of the CBM and linker of EGLII fused to xylanase B..</i>	<i>207</i>
APPENDIX C.....	208
<i>Hypothetical digestion of the CBM+linker+xylanase B with chymotrypsin on ExPASy</i>	<i>208</i>
<i>Hypothetical digestion of the CBM+linker+xylanase B with Trypsin on ExPASy...</i>	<i>209</i>
<i>Peak lists of peptide mass with chymotryptic digestion from MALDI-TOF analysis</i>	<i>210</i>
<i>Peak lists of peptide mass with tryptic digestion from MALDI-TOF analysis</i>	<i>212</i>

List of Figures

<i>Figure 1-1. Life cycle of <i>Aspergillus nidulans</i>.</i>	2
<i>Figure 1-2. A schematic diagram of mechanics of enzymatic hydrolysis on cellulose.</i>	6
<i>Figure 1-3. A hypothetical model of the tertiary structure of CBHI.</i>	8
<i>Figure 1-4. Modular structures of CBHII, EGLII and XYNII.</i>	10
<i>Figure 1-5. A typical tertiary structure of family 11 xylanase from <i>Trichoderma reesei</i>.</i>	20
<i>Figure 1-6. Putative binding sites for regulatory factors in the <i>cbh1</i> promoter.</i>	28
<i>Figure 1-7. <i>Xyn2</i> promoter region with the cis-acting and trans-acting elements and an unknown repressor.</i>	30
<i>Figure 1-8. N-glycosylation pathways in mammals and yeast.</i>	45
<i>Figure 3-1. Schematic diagram of all gene expression cassettes with detailed internal design features including proteolytic cleavage sites.</i>	64
<i>Figure 3-2. Schematic representation of the assembly of the gene expression vector <i>pXYN2sigpro</i> in <i>pUC19</i>.</i>	67
<i>Figure 3-3. Schematic representation of all gene expression plasmids assembled in <i>pUC19</i>; <i>pXYN2sigpro</i>, <i>pCBH2sigpro</i>, <i>pCBH2cbmlin</i>, <i>pEGL2sigpro</i> and <i>pEGL2cbmlin</i>.</i>	70
<i>Figure 3-4. Culture supernatants from cultivations with the starting pH of 7.0 and 6, separated on SDS-PAGE.</i>	71
<i>Figure 3-5. Images of 2D SDS-PAGE gels from the culture-supernatants.</i>	75
<i>Figure 4-1. Overview of the screening procedure for recombinant <i>T. reesei</i> strains generated with the gene expression cassettes, <i>XYN2sigpro</i>, <i>CBH2sigpro</i>, and <i>CBH2cbmlin</i>.</i>	86
<i>Figure 4-2. (1) An example of detection of Xylanase B activity on ALS/ agarose/ xylan (2) Xylanase assay with heat-treated, cleared culture supernatants.</i>	89
<i>Figure 4-3. (1) SDS-PAGE gel of the key XS strains (2) a zymogram conducted using the SDS-PAGE gel.</i>	90
<i>Figure 4-4. (1) SDS-PAGE gel of the key ES strains (2) a zymogram conducted using the SDS-PAGE gel.</i>	91
<i>Figure 4-5. (1) SDS-PAGE gel of the key EC strains. (2) a zymogram conducted using the SDS-PAGE gel.</i>	92
<i>Figure 4-6. Schematic representation of the cleavage of the gene product in transformants carrying the gene expression cassette <i>CBH2cbmlin</i>.</i>	93
<i>Figure 4-7. (1) SDS-PAGE gel of the key CS isolates. (2) a zymogram conducted using the SDS-PAGE gel.</i>	94
<i>Figure 4-8. (1) SDS-PAGE gel of some CC recombinants. (2) a zymogram conducted using the SDS-PAGE gel.</i>	95
<i>Figure 4-9. Overall comparison of the selected strains from all five groups of transformants containing either <i>XYN2sigpro</i> (XS), <i>CBH2sigpro</i> (CS), <i>CBH2cbmlin</i> (CC), <i>EGL2sigpro</i> (ES), or <i>EGL2cbmlin</i> (EC).</i>	99
<i>Figure 4-10. A schematic representation of the detection of homologous integration of the transforming DNA in the <i>CBH2sigpro</i> and <i>CBH2cbmlin</i> transformants.</i>	102
<i>Figure 4-11. A photograph of the film from a Southern blot hybridised with the 308 bp <i>xynB</i> probe.</i>	103
<i>Figure 4-12. Overall comparison of transformants containing the <i>XYN2sigpro</i> (XS) vector graphed in terms of gene copies, homologous integration events and Xylanase B activity.</i>	110
<i>Figure 5-1. Overview of the screening procedure for multiple-promoter transformants generated with the gene expression cassettes <i>XYN2sigpro</i> and <i>CBH2cbmlin</i> in combination.</i>	115

Figure 5-2. Fragments created with restriction enzymes for the detection of the <i>xyn2</i> , <i>egl2</i> and <i>cbh2</i> promoters of <i>T. reesei</i> RUT-C30.....	118
Figure 5-3. Total catalytic activity of Xylanase B obtained from 3.5 ml induction cultures of the 24 transformants.....	120
Figure 5-4. Primers binding to the <i>XYN2sigpro</i> ; (A), <i>CBH2cbmlin</i> ; (B) and <i>EGL2cbmlin</i> ; (C) gene cassettes.....	122
Figure 5-5. Proteins secreted by selected multiple-promoter transformants, displayed on 12 % SDS-PAGE gels.....	124
Figure 5-6. Reducing sugars produced from beechwood xylan by enzymatic activity of Xylanase B measured in mM from a dilution series of heat-denatured, cleared supernatants from cultures of MPP-4, -6, -7, -8, -10, -13, and -14.....	126
Figure 5-7. Xylanase B activity calculated in nkat/ml with MPP-4, -6, -7, -8, -10, -13, and -14.....	127
Figure 5-8. Reducing sugars produced by enzymatic activity of Xylanase B.....	128
Figure 5-9. Xylanase B activity expressed as nkat/ml from strains MPP-1, -3, and -4 and EC-21.....	128
Figure 5-10. Schematic for detection of <i>XYN2sigpro</i> and <i>CBH2cbmlin</i> gene cassettes incorporated into the homologous <i>xyn2</i> and <i>cbh2</i> loci in the multiple-promoter (MPP) transformants.....	130
Figure 5-11. Detection of the <i>xyn2</i> , <i>cbh2</i> and <i>egl2</i> promoter regions in the gDNA of the selected MPP transformants digested with given restriction enzymes.....	132
Figure 5-12. Amplification of the <i>EGL2cbmlin</i> cassette from the gDNA of the EC-21 transformant.....	136
Figure 5-13. Reducing sugars produced from xylan in mM by Xylanase B in a five minute assay.....	139
Figure 5-14. Comparison of the Xylanase B activity produced by the best single- and multiple-promoter strains.....	140
Figure 6-1. A flowchart for the strategies used for characterisation of the isoforms of Xylanase B expressed by the EC-21 and MPP-4 transformants.....	145
Figure 6-2. (A) Proteins from heat-treated culture supernatants separated on an SDS-PAGE gel before the zymogram procedure. (B) The zymogram.....	149
Figure 6-3. Fusion protein produced from EC-21 and MPP-4 carrying the gene expression cassette, <i>EGL2cbmlin</i>	151
Figure 6-4. A schematic diagram of the recombinant Xylanase B fused with the CBM and the linker fragments of <i>EGLII</i>	153
Figure 6-5. An SDS-PAGE gel of the heat treated and concentrated supernatants of EC-21 and MPP-4.....	154
Figure 6-6. Spectra of masses of N- and O-linked glycans released from the EC-21 sample and identified by LC-ESI-MS.....	156
Figure 6-7. A spectrum of the peaks resulting from fragmentation by MS/MS of the peak 843.3.....	158
Figure 7-1. Summary of all transformants and their lineages.....	165

List of Tables

Table 1-1. Commercial enzyme products and their industrial applications	3
Table 1-2. Identified <i>T. reesei</i> enzymes degrading biomass	7
Table 1-3. Characteristics of commercially-applied expression systems for recombinant protein production	11
Table 1-4. Potential applications of xylanases covering food, feed and technical sectors.	18
Table 1-5. Commercially available xylanase products	19
Table 1-6. Yields of heterologous gene products expressed under the <i>T. reesei</i> <i>cbh1</i> promoter	27
Table 2-1. Solutions frequently used in this research.	49
Table 2-2. Chemicals used for the preparation of acetamide agar	52
Table 2-3. Frequently used programmes in the GCG package and their functions.	53
Table 2-4. Primers used for the amplification of the DNAs applied as hybridisation probes	55
Table 3-1. Identification of the genes coding for CBHII, EGLII and XYNII.....	63
Table 3-2. Primers used for the construction of gene expression vectors.....	65
Table 3-3. Identification of the protein-spots by MALDI-TOF-MS.....	77
Table 3-4. Detailed information on CBHII, EGLII and XYNII of <i>T. reesei</i>	80
Table 4-1. A summary of the expression cassettes used for transformation	88
Table 4-2. Transformation efficiency with five different expression vectors	97
Table 4-3. Xylanase B activity of all selected transformants representing XS-XYN2sigpro, CS-CBH2sigpro, CC-CBH2cbmlin, ES-EGL2sigpro and EC-EGL2cbmlin cassettes	98
Table 4-4. Gene copy number and DNA integration analysis with CBH2sigpro (CS) and CBH2cbmlin (CC) transformants.....	103
Table 4-5. Gene copy number and DNA integration analysis with EGL2sigpro (ES), EGL2cbmlin (EC), and XYN2sigpro (XS).....	104
Table 4-6. A summary of the gene copies and the event of homologous integration of all transformants carrying the XYN2sigpro gene expression cassette	105
Table 4-7. Comparison of the gene copy numbers and DNA integration in the selected CBH2sigpro, CBH2cbmlin, EGL2sigpro and EGL2cbmlin transformants.....	108
Table 4-8. An overall comparison of XYN2sigpro transformants with respect to gene copies, homologous integration events and Xylanase B activity	109
Table 5-1. Primers used to check homologous integration of the gene expression cassettes in the multiple-promoter transformants by PCR.....	117
Table 5-2. A summary of the multistage selection process of transformants.	119
Table 5-3. Xylanase B activity obtained from 3.5 ml induction cultures with selected transformants	121
Table 5-4. A summary of the combination of promoters detected in the multiple-promoter transformants	123
Table 5-5. Xylanase B activity of the MPP transformants in nkat/ml.....	127
Table 5-6. Xylanase B activity of the selected MPP-transformants and the single promoter strain EC-21	129
Table 5-7. Approximation of the number of <i>xyn2</i> and <i>cbh2</i> gene promoter sequences in selected MPP transformants	134
Table 5-8. Comparison of Xylanase B activity between MPP-4 and selected single-promoter strains	140
Table 5-9. Increases in Xylanase B activity from single- to multiple-promoter strains. .	141
Table 6-1. Assigned compositions of O- and N-linked sugars based on the LC-ESI-MS/MS spectra.....	157

Abstract

Filamentous fungi are attractive as cell factories for recombinant protein production. *Trichoderma reesei*, an industrially-important filamentous fungus, has been used for the expression of recombinant gene products for over a decade and a variety of molecular genetics strategies has been applied to increase the yields of these products; yet no substantial improvements have been made and a significant increase in the yields remains a priority for economic production of non-fungal recombinant proteins. In this work, a new approach was developed to increase further recombinant protein production in *T. reesei*, featuring simultaneous use of multiple promoters for gene expression. Reliance on a single strong promoter, such as the one from the *cbh1* gene encoding the main cellobiohydrolase I (CBHI), for driving expression of the genes encoding proteins of interest may not utilise the full capacity of protein expression by the organism. Hence, the novel concept of a multiple promoter platform (MPP) was put into practice with the objective of improving the efficiency of recombinant (heterologous) protein production. Instead of using one type of promoter for gene expression, the strategy behind MPP was to increase overall transcription of the gene of interest, here *xynB* encoding a thermophilic bacterial xylanase enzyme (Xylanase B), by employing multiple different fungal gene promoters.

Enhancement of heterologous protein production was explored by combined proteomic, molecular genetic and glycoproteomic approaches. Major secreted proteins from *T. reesei*, including EGLII, CBHII and XYNII, were identified by 2-DE and MALDI-TOF analyses and the promoter sequences of the genes encoding these proteins were used to construct expression vectors for the production of the heterologous Xylanase B (XynB) from the thermophilic bacterium, *Dictyoglomus thermophilum*. Two generations of transformants carrying either a single promoter (SP) or multiple promoters (MP) controlling the expression of the *xynB* gene were examined. The best performing SP and MP recombinants were selected based on Xylanase B activity. The increase of activity in the best strain carrying the three different promoters to drive the expression of the *xynB* gene was 170 % compared to that of the best SP strain (transformation host for the MP strains). The copy number of the integrated genes and the mode of integration of the expression cassettes by homologous or non-homologous recombination were not significant factors in the production of Xylanase B, suggesting the presence of additional, as yet unidentified elements were involved in heterologous protein production. Examination of the glycosylation of Xylanase B fused to the linker region of EGLII revealed considerable

amounts of O-linked oligosaccharides attached to the protein. One of the sugars identified by LC-MS/MS was hexuronic acid, which presents a sugar not previously found in *Trichoderma* glycoproteins. Overall, production of Xylanase B was enhanced by using a combination of three promoters, *xyn2*, *cbh2* and *egl2* inducing *xynB* gene expression.

Declaration

The research presented in this thesis is original work conducted between March 2006 and October 2009 by the author. This material has not been submitted as part of the requirement for any other degree or course to any other institution. To the best of my knowledge it contains no material previously published or written by any other person except where due reference is made in the text.

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Abbreviations

Abbreviations frequently used in the text are:

Amp	Ampicillin resistance
BSA	Bovine serum albumin
bp	Base pairs
<i>cbh2</i>	Gene coding CBHII enzyme
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleoside triphosphates
EDTA	Ethylenediamine-tetra-acetic acid
<i>egl2</i>	Gene encoding EGLII enzyme
ER	Endoplasmic reticulum
g	Specific gravity
IPTG	Isopropyl- β -D-thiogalactopyranoside
kb	Kilobase pairs
kDa	Kilodalton
<i>lacZ</i>	β -galactosidase gene
LB	Luria Broth
mRNA	Messenger ribonucleic acid
M	Moles per litre
min	Minute
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
RNA	Ribonucleic acid
rpm	Revolutions per minute
SAP	Shrimp alkaline phosphatase
SDS	Sodium dodecyl sulphate
TBE	Tris-borate-EDTA
Tris	[2-amino-2-(hydroxymethyl)propane-1,3-diol, (tris)]
UV	Ultra violet
V	Volt
v/v	Volume per volume
v/w	Volume per weight
w/w	Weight per weight
X-Gal	5-bromo-4-chloro-3-indolyl β -D-galactopyranoside
<i>xyn2</i>	Gene encoding xylanase II enzyme