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Gene Cloning and Characterisation of a New Glycosyl Hydrolase Family 3 β-Glucosidase from *Aspergillus terreus* SUK-1

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Abstract: A reverse transcriptase-polymerase chain reaction was used to isolate a gene for β-glucosidase from fungi *Aspergillus terreus* SUK-1. The DNA consisted of 2586 nucleotides coding sequence encoding 861 amino acids for BGat1 protein. Both nucleotide and amino acid sequences possessed high similarity match with reported β-glucosidase genes and proteins such as of *Aspergillus* spp. and *Neosartorya fischeri*. The presence of a 19 amino acids leader sequence suggested that BGat1 is an extracellular protein and very conserve with 5 other β-glucosidases tested. Two specific domains, namely GHF3 N terminal domain and GHF3 C terminal domain, were detected in BGat1 and these domains were separated by a spacer of 99 amino acids. This suggests that BGat1 is a member of glycosyl hydrolase superfamily 3 proteins. Expression analysis showed that *BGat1* gene was induced as early as 7 hours after fungal incubation and the level of gene expression maintained at almost the same until day 7. *BGat1* expression was also detected when *A. terreus* SUK-1 was grown in the presence of glucose suggesting that the gene was not inhibited by the product of enzymatic reaction.

Key words: *Aspergillus terreus* SUK-1, β-glucosidase, cellulase, glucose, expression

INTRODUCTION

Cellulose is one of the most important enzymes in industrial applications and comprises of 3 enzyme substitutes namely cellobiohydrolase, endoglucanase and β-glucosidase. These 3 enzymes work synergistically to degrade cellulose polymer into very important glucose molecules. Endoglucanase is the first enzyme to react randomly on amorphous region loosening cellulose structure. Cellobiohydrolase then attack cellulose polymer at the ends producing less complex molecules such as cellobiose. Finally, β-glucosidase acts on cellobioses releasing glucose molecules, while β-glucosidase acts on cellobioses to finally release glucoses (Tomme *et al*., 1995; Teeri *et al*., 1998). Products of cellulase degradation are used in many commercially important industries. The final product of glucose is one of the most important precursors for biofuel (Wilson, 2009; Sims *et al*., 2010) and brewing industries (Bhat 2000; Nomachi *et al*., 2002). Cellulase enzymatic reaction on fabric softens the cellulose physical characteristic and very important in textile industry (Ibrahim *et al*., 2005). Plant biomass cellulose degradation is important in pulp and paper industry (Ferraz *et al*., 2008; Zhang *et al*., 2008) and fertilizer industry (Han and He 2010). In food processing, cellulase is used in the extraction and clarification of fruit and vegetable juices (Galante *et al*., 1998; Grassin and Fauquembergue, 1996).

In order to live, many organisms produce cellulase as an inducible enzyme for substrate degradation. Studies showed that cellulase from fungi possessing better cellulosic enzymatic activity compared to the one from bacterial. As such, many commercially available cellulases have been produced mainly from *Trichoderma* sp. and *Aspergillus* sp. (Godfrey and West, 1996; Uhlig, 1998). It was reported that cellulase accounts for more than 20% of the world enzyme market (Mantyla *et al*., 1998). However, high demand on a better and more efficient cellulosic enzymatic cellulase requires searching for a new and better enzyme. In our study, *A. terreus* SUK-1 isolated from oil palm industry effluent was used. Earlier characterisation by our group indicated that cellulase produced by *A. terreus* SUK-1 possessing a better and more reactive enzyme compared to some commercially available cellulases (Omar *et al*., 1984; Kader *et al*., 1988). In order to further characterise the enzyme behavior at molecular level, cellulase genes were isolated. Previously we described the isolation and molecular characterisation of cellobiohydrolase (Sidik *et al*., 2011a) and endoglucanase (Sidik *et al*., 2006; Sidik *et al*., 2011b) from *A. terreus* SUK-1. This paper describes the isolation and characterisation of a new β-glucosidase gene and its expression characteristic.

MATERIALS AND METHODS

Organism and Growth Condition:

Aspergillus terreus SUK-1 used in this research was obtained from Professor Dr. Othman Omar from Universiti Kebangsaan Malaysia, Bangi, Malaysia. Throughout the research period, the culture was grown and

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maintained on Potato Dextrose Agar (PDA). For RNA extraction purpose, *A. terreus* SUK-1 was grown in Mandel medium as described by Sidik *et al*. (2011a).

Total RNA Isolation and cDNA Synthesis:

Cells were harvested by filtration using sterile pieces of nylon gauze. The cells were then frozen and ground with a mortar and pestle under the presence of liquid nitrogen. Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen, Germany) according to the supplier's recommendation. 3 µg of total RNA was used to generate first strand cDNA using SuperScript™ cDNA Synthesis Systems (Invitrogen, USA) as described by Sidik *et al*. (2011a).

Gene Isolation and Cloning:

A PCR-based approach was used to clone the gene. A pair of primer was design based on conserved region of β-glucosidase available in the GeneBank. The forward primer was BGatF1 (5'-ATGAAGCTTTCCATTTTGGAGGCA-3') and the reverse primer was BGatR1 (5'-TTACTGCACCCGTGGCA GCG-3'). 50 ng of the first strand cDNA were used for gene amplification with PCR. PCR amplification was done by the following cycling condition; one pre-denaturation cycle at 94 °C for 3 minutes; 30 cycles at 94 °C for 30 seconds, 58 °C for 60 seconds, 72 °C for 3 minutes followed by a final extension cycle at 72°C for 10 minutes. PCR product was analysed on 1 % agarose gel, stained with ethidium bromide and visualised under UV light. Band of interest was purified from gel as described by Mohd. Fahmi *et al*. (2010). The purified PCR fragment was cloned into pCR®II-TOPO plasmid vector and transformed into *E. coli* DH5*α* as recommended by the supplier (Invitrogen, USA). Plasmid purification was done using QIAprep Spin Miniprep Kit (Qiagen, Germany) according to the supplier's recommendation.

DNA Aequencing and Snalyses:

DNA was sent to the 1st Base Pte. Ltd., Singapore for sequencing. DNA sequences were edited with BIOEDIT (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequence analyses were done using Blast programme (http://www.ncbi.nlm.nih.gov/BLAST/) and NCBI Conserved Domain Search for similarity search. DNA sequences were also scanned against Protein Families of Alignments (Pfam) (http://www.sanger.ac.uk/software/pfam) for possible protein conservation. Analysis for gene prediction was done using GENSCAN (http://gene.mit.edu/GENSCAN.html).

Reverse Transcription-PCR (RT-PCR) for Gene Expression Analysis:

A. terreus SUK-1 was grown in Mandel medium and total RNA was isolated 7 hours after inoculation and subsequently every 24 hours after inoculation for 7 days using RNeasy Plant Mini Kit (Qiagen, Germany) as described. Total RNA was also isolated from *A. terreus* SUK-1 grown in Mandel medium in the presence of 0.04 M glucose. First strand cDNA was synthesised as described. A reverse transcriptase-polymerase chain reaction (RT-PCR) approach using gene-specific primers was used to study the expression of *BGat1* gene. 50 ng of the first strand cDNA was used as a template for RT-PCR. The primers used for RT-PCR was the same as the one used for gene isolation. PCR amplification was done following cycling condition as used for gene isolation. PCR product was analysed on 1% agarose gel, stained with ethidium bromide and visualised under UV light. A housekeeping gene, *ef1α* for transcriptional elongation factor 1-α was also amplified as a control.

RESULTS AND DISCUSSION

A PCR approach using specific pair of primers successfully amplified a DNA fragment of 2586 nucleotides coding sequence for BGat1 gene encoding 861 amino acids (Figure 1) with estimated calculated molecular mass of 93.3 kDaltons. BGat1 nucleotide sequences showed very high similarity with endoglucanase genes various sources such as from *A. terreus* NIH2624, *A. avenaceus*, *Neosartorya fischeri* NRRL 181 and *A. aculeatus*. While BGat1 amino acids showed very high similarity with β-glucosidase from *A. terreus* NIH2624*, A. avenaceus*, *A. oryzae* RIB40 and *A. flavus* NRRL3357 (Table 1). Similarity to nucleotide and amino acid sequences were mostly to β-glucosidase from *Aspergillus* species. A predicted leader sequence of 19 amino acids was detected by SignalP 3.0 software (Bendtsen *et al*., 2004) leaving a predicted mature protein of 842 amino acids. Comparison of the BGat1 signal peptide with signal peptides from other β-glucosidase showed that the sequences are highly conserved (Figure 2). This suggests that, just like other β-glucosidase, BGat1is an extracellular protein.

BGat1 protein belongs to glycosyl hydrolase family 3 (GHF3). Two specific domains were present in BGat1 protein sequence. The GHF3 N terminal domain constitutes of 224 amino acids was located between Thr78 to Pro301 while the GHF3 C terminal domain of 235 amino acids was extended from Val401 and Tyr635. The GHF3 N terminal domain was predicted to serve as a catalytic domain. The 2 domains were separated by a spacer of 99 amino acids (Figure 1). The GHF3 protein is a group of enzyme that catalysed the hydrolysis of the

glycosidic bonds in carbohydrate molecules. It is part of glycosyl hydrolase superfamily which divided into at least 85 different families (Henrisat, 1991) based on their amino acid similarities. Several enzymes are classified into GHF3 such as glucosidase, xylosidase, glucosaminidase, cellodextrinase and glucanase. The special characteristic of GHF3 is having two domains located on either C terminal or N terminal which are the GHF3 C terminal domain and GHF3 N terminal domain. According to Varghese *et al*. (1999) the region between 2 domains is believed to be involved as an active site for enzymatic activity. β-glucosidase from *Physarum polycephalum* (Hayase *et al*., 2008), *Periconia* sp. (Hampichamchai *et al*., 2009), *Thermotoga neapolitana* (Pozzo *et al*., 2010) and *Penicilium pinophilum* (Joo *et al*., 2010) are classified into the same group as BGat1. However, not all β-glucosidase proteins are classified into GHF3. β-glucosidase Cel1A of *Piromyces* sp (Harhangi *et al*., 2002) and BglU of *Micrococcus antarticus* (Fan *et al*., 2011) are grouped into GHF1, whereas, β-glucosidase from *A. oryzae* is classified under GHF12 (Kitamoto *et al*., 1996).

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ATGAAGCTTTCCATTTTGGAGGCAGCAGCTTTGACAGCTGCCTCCGTCGCCAGCGCACAGGACGATCTCGCATACTCCCCGCCGTACTAC      90
 M  K  L  S  I  L  E  A  A  A  L  T  A  A  S  V  A  S  A  Q  D  D  L  A  Y  S  P  P  Y  Y       30
CCTTCTCCCTGGGCCGATGGCCACGGTGAGTGGTCGAACGCGTACAAACGCGCCGTAGATATCGTCTCTCAGATGACATTGACGGAGAAG     180
 P  S  P  W  A  D  G  H  G  E  W  S  N  A  Y  K  R  A  V  D  I  V  S  Q  M  T  L  T  E  K       60
GTCAATCTCACCACCGGTACTGGATGGGAGTTGGAGAGGTGTGTCGGTCAGACGGGCAGTGTCCCTAGACTGGGGATTCCAAGCCTCTGT     270
 V  N  L  T  T  G  T  G  W  E  L  E  R  C  V  G  Q  T  G  S  V  P  R  L  G  I  P  S  L  C       90
TTGCAGGATAGCCCTCTGGGTATTCGCATGTCGGACTATAACTCGGCCTTCCCTGCGGGTATTAACGTTGCGGCCACCTGGGACAAGACG     360
L  Q  D  S  P  L  G  I  R  M  S  D  Y  N  S  A  F  P  A  G  I  N  V  A  A  T  W  D  K  T      120
CTTGCCTACCAACGCGGCAAGGCAATGGGCGAGGAATTCAGTGACAAGGGTATTGATGTTCAGTTGGGCCCTGCTGCCGGTCCTCTTGGC     450
L  A  Y  Q  R  G  K  A  M  G  E  E  F  S  D  K  G  I  D  V  Q  L  G  P  A  A  G  P  L  G      150
AGGTCCCCCGATGGAGGCCGAAACTGGGAGGGCTTCTCTCCTGATCCCGCCCTGACTGGTGTGTTGTTCGCCGAGACGATCAAGGGTATC     540
R  S  P  D  G  G  R  N  W  E  G  F  S  P  D  P  A  L  T  G  V  L  F  A  E  T  I  K  G  I      180
CAGGACGCCGGAGTTATTGCTACCGCGAAACACTACATTCTCAACGAACAAGAGCATTTCCGCCAGGTCGGCGAAGCCCAGGGCTATGGC     630
Q  D  A  G  V  I  A  T  A  K  H  Y  I  L  N  E  Q  E  H  F  R  Q  V  G  E  A  Q  G  Y  G      210
TTCAACATCACCGAAACTGTGAGCTCGAATGTGGATGACAAAACCATGCACGAGCTGTATCTCTGGCCCTTCGCCGATGCGGTGCGCGCG     720
F  N  I  T  E  T  V  S  S  N  V  D  D  K  T  M  H  E  L  Y  L  W  P  F  A  D  A  V  R  A      240
GGCGTGGGCGCTGTGATGTGCTCCTATAACCAGATCAACAACAGCTACGGATGCCAAAACAGTTTAACCCTGAACAAGCTCTTGAAAGCC     810
G  V  G  A  V  M  C  S  Y  N  Q  I  N  N  S  Y  G  C  Q  N  S  L  T  L  N  K  L  L  K  A      270
GAACTCGGATTTCAGGGATTTGTCATGAGTGACTGGAGTGCTCACCACAGCGGTGTTGGCGCCGCCTTGGCTGGTTTGGACATGTCCATG     900
E  L  G  F  Q  G  F  V  M  S  D  W  S  A  H  H  S  G  V  G  A  A  L  A  G  L  D  M  S  M      300
CCGGGAGATATCAGCTTCGACAGCGGCACTTCCTTCTATGGCACGAACTTGACTGTTGGCGTCCTCAACGGCACCATTCCCCAGTGGCGT     990
P  G  D  I  S  F  D  S  G  T  S  F  Y  G  T  N  L  T  V  G  V  L  N  G  T  I  P  Q  W  R      330
GTGGATGATATGGCCGTCCGGATCATGGCTGCCTACTACAAGGTTGGCCGCGACCGTCTCTGGACCCCTCCCAATTTCAGCTCGTGGACT    1080
 V  D  D  M  A  V  R  I  M  A  A  Y  Y  K  V  G  R  D  R  L  W  T  P  P  N  F  S  S  W  T      360
CGCGATGAATATGGCTTCGCGCACTTCTTCCCTTCCGAAGGCGCTTATGAACGTGTCAATGAATTTGTCAACGTGCAGCGTGACCATGCC    1170
 R  D  E  Y  G  F  A  H  F  F  P  S  E  G  A  Y  E  R  V  N  E  F  V  N  V  Q  R  D  H  A      390
CAGGTGATCCGTCGGATTGGCGCGGATAGTGTCGTGCTCTTGAAGAACGACGGTGCCCTTCCCTTGACGGGCCAGGAAAAGACTGTTGGC    1260
 Q  V  I  R  R  I  G  A  D  S  V  V  L  L  K  N  D  G  A  L  P  L  T  G  Q  E  K  T  V  G      420
ATTCTGGGCGAAGACGCTGGATCGAATCCGAAGGGAGCAAACGGTTGCAGTGACCGCGGCTGTGACAAGGGTACTTTGGCCATGGCTTGG    1350
 I  L  G  E  D  A  G  S  N  P  K  G  A  N  G  C  S  D  R  G  C  D  K  G  T  L  A  M  A  W      450
GGTAGTGGTACTGCCAACTTCCCTTACCTTGTGACTCCCGAACAGGCCATTCAGAACGAGGTTCTGAAGGGCCGTGGAAATGTCTTTGCC    1330
 G  S  G  T  A  N  F  P  Y  L  V  T  P  E  Q  A  I  Q  N  E  V  L  K  G  R  G  N  V  F  A      480
GTGACGGACAACTATGATACGCAGCAGATTGCCGCCGTTGCCTCTCAATCCACGGTTTCGTTGGTTTTCGTGAATGCGGACGCAGGTGAA    1530
 V  T  D  N  Y  D  T  Q  Q  I  A  A  V  A  S  Q  S  T  V  S  L  V  F  V  N  A  D  A  G  E      510
GGTTACCTTAATGTGGACGGAAACATGGGTGATCGCAAGAACCTCACCCTCTGGCAGAACGGAGAGGAAGTGATCAAGACCGTCACGGAG    1620
 G  Y  L  N  V  D  G  N  M  G  D  R  K  N  L  T  L  W  Q  N  G  E  E  V  I  K  T  V  T  E      540
CACTGCAACAACACCGTCGTTGTGATCCATTCGGTGGGACCTGTTCTCATCGATGAGTGGTATGCGCACCCCAATGTCACCGGCATTCTG    1710
 H  C  N  N  T  V  V  V  I  H  S  V  G  P  V  L  I  D  E  W  Y  A  H  P  N  V  T  G  I  L      570
TGGGCTGGTCTCCCGGGCCAGGAGTCTGGCAACGCCATTGCGGACGTGCTGTACGGCCGCGTCAACCCTGGCGGCAAGACCCCCTTTACC    1800
 W  A  G  L  P  G  Q  E  S  G  N  A  I  A  D  V  L  Y  G  R  V  N  P  G  G  K  T  P  F  T      600
TGGGGTAAGACGCGCGCGTCCTACGGCGACTACCTCCTCACCGAGCCCAACAACGGCAACGGTGCTCCTCAAGACAACTTCAACGAGGGC    1890
 W  G  K  T  R  A  S  Y  G  D  Y  L  L  T  E  P  N  N  G  N  G  A  P  Q  D  N  F  N  E  G      630
GTGTTTATCGACTACCGTCGCTTCGACAAGTACAATGAGACACCCATCTACGAGTTCGGCCATGGTCTGAGCTACACGACTTTTGAACTG    1980
 V  F  I  D  Y  R  R  F  D  K  Y  N  E  T  P  I  Y  E  F  G  H  G  L  S  Y  T  T  F  E  L      660
TCTGGCCTCCAGGTTCAGCTTATCAACGGATCCAGCTATGTTCCCACTACGGGTCAGACGAGCGCCGCCCAGACATTTGGTAAAGTCGAG    2070
 S  G  L  Q  V  Q  L  I  N  G  S  S  Y  V  P  T  T  G  Q  T  S  A  A  Q  T  F  G  K  V  E      690
GACGCGTCTAGCTACCTGTACCCGGAGGGACTGAAGAGAATTTCCAAGTTCATCTATCCCTGGCTGAACTCTACCGATCTTAAAGCGTCT    2160
 D  A  S  S  Y  L  Y  P  E  G  L  K  R  I  S  K  F  I  Y  P  W  L  N  S  T  D  L  K  A  S      720
ACTGGCGATCCTGACTACGGAGAGCCCAACTTCGAGTATATCCCTGAAGGAGCTACCGATGGCTCTCCTCAGCCCCGTCTGCCTGCCAGC    2250
 T  G  D  P  D  Y  G  E  P  N  F  E  Y  I  P  E  G  A  T  D  G  S  P  Q  P  R  L  P  A  S      750
GGGGGTCCTGGCGGCAACCCCGGTCTCTACGAGGATCTGTTCCAGGTTTCTGTGACCATCACCAACACCGGCAAGGTTGCTGGTGATGAG    2340
 G  G  P  G  G  N  P  G  L  Y  E  D  L  F  Q  V  S  V  T  I  T  N  T  G  K  V  A  G  D  E      780
GTGCCTCAGCTGTATGTTTCGCTGGGTGGCCCCAACGAGCCGAAGCGGGTGCTGCGCAAGTTTGAGCGTCTGCACCTCGCCCCTGGTCAG    2430
 V  P  Q  L  Y  V  S  L  G  G  P  N  E  P  K  R  V  L  R  K  F  E  R  L  H  L  A  P  G  Q      810
CAGAAGGTCTGGACGACTACCCTGAACCGCCGTGACCTGGCCAACTGGGATGTTGTGGCCCAGGACTGGAAGATCACTCCCTATGCTAAG    2520
 Q  K  V  W  T  T  T  L  N  R  R  D  L  A  N  W  D  V  V  A  Q  D  W  K  I  T  P  Y  A  K      840
ACCATCTTTGTTGGCACCTCTTCACGCAAACTGCCTCTCGCTGGTCGCTTGCCACGGGTGCAGTAA                            2586
 T  I  F  V  G  T  S  S  R  K  L  P  L  A  G  R  L  P  R  V  Q  *                              861
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Fig. 1: Nucleotide and deduced amino acid sequences of the *BGat1* gene. Numbers on the right refer to nucleotide and amino acid sequences. A putative signal peptide sequence is underlined. The glycosyl hydrolase family 3 (GHF3) C terminal domain is indicated by bold letters. The GHF3 N terminal domain is indicated by italic letters. Asterisk (*) indicates stop codon. The GeneBank accession number for the sequence is GU078571.1.

Expression analysis showed that *BGat1* gene was constitutively expressed. *BGat1* was expressed as early as 7 hours after incubation and expression level maintained at almost the same until day 7. Addition of glucose to the growth media did not affect the gene expression (Figure 3). Although many studies showed that β-glucosidase was suppressed by glucose, exception occurs on β-glucosidases produced by *Aspergillus* species (Li and Lee, 1999; Murray *et al*., 2004; Singhania *et al*., 2011). *A. terreus* SUK-1 requires glucose for living as it serves as the primary sources for carbon an energy. In the media, the carbon source was supplied in the form of α-cellulose. Cell's endoglucanase enzyme hydrolyses the cellulose into two glucose molecules cellobiose and

the cellobiose will further be hydrolysed by β-glucosidase into useable simple carbohydrate molecule of glucose. In active growing cells, genes for the both enzymes are highly expressed. In *A. terreus* SUK-1, endoglucanase gene was expressed and reached maximum level just after 7 hours after incubation in order to produce enough amount of enzyme for cellulose degradation. As cellobiose continually produced and reached threshold level endoglucanase gene is suppressed (Sidik *et al*. 2011a). However, *BGat1* was constitutively expressed. We believed that β-glucosidase enzyme is constitutively required to maintain enough supply of glucose to the cells. Therefore, β-glucosidase is not catabolite repressed by glucose. Catabolite repression of β-glucosidase gene by glucose may cause limited supply of energy source to the cell and subsequently inhibit cell growth and replication.

Table 1: BLAST analyses of the *BGat1* nucleotide and amino acid sequences.

Gene/Protein	Identity (%)	Organism	GenBank accession number
Gene			
β-glucosidase 1	98	*A. terreus* NIH2624	XM_001212225.1
β-glucosidase *bgl1*	97	*A. avenaceus*	AY943971.1
β-glucosidase	76	*N. fischeri* NRRL 181	XM_001265090.1
β-glucosidase	76	*A. aculeatus*	D64088.1
β-glucosidase	76	*A. fumigatus* Af293	XM_745234.1
Protein			
β-glucosidase 1	99	*A. terreus* NIH2624	XP_001212225.1
β-glucosidase	96	*A. avenaceus*	AAX39011.1
β-glucosidase A	82	*A. oryzae* RIB40	XP_001816831.1
β-glucosidase	82	*A. flavus* NRRL3357	XP_002383240.1
β-glucosidase BGL3	80	*A. fumigatus*	ADX78143.1
β-glucosidase A	80	*A. fumigatus* A1163	BOXPE1.1
β-glucosidase	80	*A. fumigatus* Af293	XP_750327.1
β-glucosidase 1	79	*A. aculeatus*	P48825.1
β-glucosidase GH3	77	*Aspergillus* sp. AS-2011	AEL79685.1
β-glucosidase	79	*N. fischeri* NRRL 181	XP_00126509.1
β-glucosidase	79	*A. clavatus* NRRL1	XP_001269582.1
β-glucosidase A	77	*A. niger* CBS 513.88	XP_001398816.1
β-glucosidase	77	*A. niger*	CBA02054.1
β-glucosidase II	77	*A. niger*	ACV91073.1

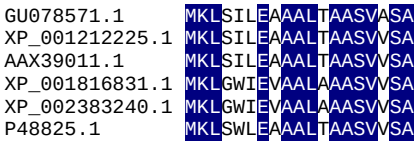

Fig. 2: Alignment of BGat1 signal peptide with 5 other signal peptides of β-glucosidase from filamentous fungi. Identical amino acids are in reverse colour. GU078571.1, β-glucosidase BGat1 from *A. terreus* SUK-1; XP_001212225.1, β-glucosidase from *A. terreus* NIH2624, AAX39011.1, β-glucosidase from *A. avenaceus*; XP_001816831.1, β-glucosidase A from *A. oryzae* RIB40; XP_002383240.1, β-glucosidase from *A. flavus* NRRL3357; P48825.1, β-glucosidase 1 from *A. aculeatus*.

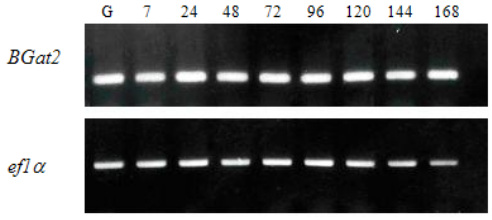

Fig. 3: RT-PCR results of expression study. Labels on the top indicate time (hour) after incubation. G, *A. terreus* SUK-1 grew with the presence of glucose. *ef1α*, transcriptional elongation factor 1-α gene as an internal control for RT-PCR.

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Conclusion:

We have isolated a new β-glucosidase gene from *A.terreus* SUK-1 namely *BGat1*. Protein sequence analysis showed that BGat1 protein belongs to the glycosyl hydrolase superfamily 3 protein. *BGat1* gene was constitutively expressed during 7 days period of study and was not inhibited by glucose.

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