African Journal of Microbiology Research Vol. 6(12), pp. 2909-2917, 30 March, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1320
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Isolation of the encoding gene for a thermostable α-glucosidase from *Geobacillus stearothermophilus* strain RM and its expression in *Escherichia coli*

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Accepted 6 February, 2012

High temperature catalysis increases substrate solubility and carbohydrate hydrolysis, therefore investigating new thermostable α-glucosidase is an attractive option. A total of seven isolates were obtained from two different hot spring locations in Malaysia, namely Telaga Air Hangat Langkawi and Slim River. Preliminary study on four selected isolates revealed that production media 1 (PM 1) and production media 2 (PM 2) were the best media to support α-glucosidase production. The optimum growth temperatures were 50-70°C while their optimum temperature for α-glucosidase production was 55°C. Quantitative screening indicated that isolate L3 which was identified as *Geobacillus stearothermophilus* strain RM was the best α-glucosidase producer with 1.47 U/ml at 55°C after 72 h. The production of α-glucosidase was also found to be growth associated up to stationary phase. Identification on the basis of morphological characteristics, biochemical studies and 16S rRNA analysis were carried out and 16S rRNA identification revealed that this isolate showed 99% similarity to *G. stearothermophilus*. The α-glucosidase gene had been successfully amplified from this identified bacterium via degenerate primer and the complete gene was cloned and expressed into *Escherichia coli* with 5 U/ml activity which was 5-folds higher compared to the wild type. As a conclusion, *E. coli* system successfully increased the yield of α-glucosidase production.

Key words: α-glucosidase*, Geobacillus stearothermophilus,* growth optimization.

INTRODUCTION

Thermophiles are defined as microorganisms that thrive at relatively high temperatures, between 45 and 80°C (Brock, 1986). As their cellular components, proteins in thermophiles are inherently more heat stable compared to those from conventional mesophilic microorganisms. This thermal stability is not due to any specific characteristic but resulted from a consequence of various changes that contribute to the whole stability of the protein in additive manner hence attributed to the probability of increasing their enzyme activity by means of genetic manipulation. Therefore, these microorganisms were the first candidates for massive enzyme production for industrial applications (Vieille, 1996).

Recent developments in the production of high-fructose syrups from starch have created further interest in commercially suitable glycoside hydrolases enzymes

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Abbreviation: PM, production media; **α-MUG,** - 4-Methylumbelliferyl-a-D glucoside.

such as α-amylase, β-amylase, glucoamylase, α-glucosidase and other enzymes. α-Glucosidases (EC 3.2.1.20) constitute a group of exo-acting glycoside hydrolases of diverse specificity that catalyse the release of α-D-glucose from the non-reducing end of α-linked substrates. Although α-glucosidase can be derived from plants and animals, the enzymes derived from microbial sources are generally used to meet the expanding industrial demands (Gupta et al., 2003). In addition to well-established applications in starch, glycogen and dextrin breakdown, α-glucosidases are also applied in textile, food, brewing, and distilling industries. Furthermore, microbial α-glucosidases could be useful for the production of pharmaceuticals, biofuels and fine chemicals (Teague and Brumm, 1992). The enzyme market showed an explosive growth from 2004 to 2009 as the US biofuels industry rapidly scaled up its production capacity and invested in new technology to meet the quotas established by the Energy Independence and Security Act of 2007 (The Freedonia Group, 2010). Commercial products that are manufactured currently using α-glucosidase enzyme are Myozyme and Lumizyme; both for treating Pompe disease or acid maltase deficiency (Wagner, 2007). The production of enzymes from wild-type thermophiles is very low, therefore for industrial applications, it is necessary to use recombinant microorganisms for example, commercial host such as *E. coli*. Protein expression in a heterologous host has been widely applied. In protein, there are membrane proteins that are difficult to overexpress especially in heterologous host. A good host would have the ability in order to express any protein including protein that need post-translational modification and easy to manipulate, such as *E. coli*. Cloning and expression in *E. coli* system have been widely used in producing important industrial enzyme in bulk quantity.

Normally, α-glucosidase gene is found in methanogenic and thermophilic archaea and yeast but very little in bacteria. In this study, a new bacterial strain producing α-glucosidase was isolated and discussed thus make this study valuable. Hence, the expression of this α-glucosidase gene in available commercial host in order to meet the industrial demand was also reported, to increase the yield of α-glucosidase production.

MATERIALS AND METHODS

Bacterial strains and selection of -glucosidase producers

A total of seven isolates; five water samples isolated from Slim River, Perak, at 70°C (*Bacillus* sp. L2, *Geobacillus* spp., SR 38, SR 40 and SR 74) and two water samples (L3 and L4) isolated from Telaga Air Hangat, Langkawi at 60°C were screened for α-glucosidase production. They were streaked on nutrient agar (NA) and Luria Bertani (LB) agar, supplemented with starch and incubated at 37°C for 24 h. A single colony obtained from each sample was cultivated into LB (for L3 and L4 sample) while (TSB) Triptic soy broth (for *Bacillus* sp. L2, *Geobacillus* spp., SR 38, SR 40 and SR 74). Both medias were supplemented with starch 1% (w/v) at 50-70°C for 24 h. *E. coli* Top 10 was used as the cloning and expression host throughout the study. The entire tests was carried out in 3 replications.

Growth optimization

All the 7 strains were grown overnight at different temperatures ranging from 37, 55, 60, 65 and 70°C in LB and NA agar. All media were supplemented with 1% (w/v) starch for α-glucosidase screening purpose.

Plate overlay method

Colonies grown on NA and LB agar supplemented with 1% (w/v) of starch were incubated at different temperatures as stated above. After incubation for 24 h, the plates were incubated for another 3 days to allow starch hydrolysis before being tested for α-glucosidase activity. The plates were then overlaid with 0.7% (w/v) agarose dissolved in phosphate buffer (0.05 M KH_2PO4-NaOH, pH 7.0) containing 0.4 mg/ml α-MUG (α-4-Methylumbelliferyl-a-D glucoside). The overlaid substrate was allowed to solidify and the plates were then incubated at 37°C for 6 h and examined under UV light. Colonies with α-glucosidase activity exhibited fluorescence color as the formation of 4-methylumbelliferone (MU). These positive colonies in replica plates were tested for the α-glucosidase production.

Production of α-glucosidase using different synthetic growth media

α-Glucosidase production was tested in three different production media denoted as PM1 [TSB supplemented with 1% starch], PM2 [0.4% (w/v) starch, 0.4% (w/v) tryptone, 0.05% (w/v) KH_2PO_4, 0.03% (w/v) $MgSO_4.7H_2O$, 0.02% (w/v) $CaCl_2$, 0.0005% (w/v) $FeSO_4$ and 0.0002% (w/v) $MnSO_4$] and PM3 [LB supplemented with 1% (w/v) starch]. The positive α-glucosidase producing strains were grown in NB and LB broth until their absorbance was reached to an OD600 of 0.5. 1 ml culture was then inoculated into production media as stated above. The cultures were incubated at 55°C (L3 and L4), 60°C (*Geobacillus* spp.) and 70°C (L2) under shaking condition (200 rpm) for 72 h. The cultures (10 ml) were harvested at 24 h intervals and subjected to α-glucosidase assay.

α-Glucosidase assay

Each α-glucosidase producer strain, was grown overnight in the appropriate medium. The grown media were centrifuged and 1 ml of phosphate buffer (0.5 M KH_2PO_4. NaOH, pH 6.5) were added into 1 ml of the supernatant and then 1 ml of 0.1% (w/v) p-nitrophenyl-α-D-glucoside (*p*NPG) was added to start the reaction. The assay was carried out at 55, 60, 65 and 70°C in a water bath for 10 min without shaking. The reaction was stopped by the addition of 2 ml of 1 M Na_2CO_3 after 10 minutes. Standards consisted of various known concentrations of *p*NP (SigmaAldrich) in phosphate buffer and read at 400 nm by using a spectrophotometer (Amersham). One unit of α-glucosidase activity was defined as the release rate of 1 µmole of *p*-nitrophenol from *p*-nitrophenyl-α-D-glucoside per min (Aneta et al., 1992).

Identification of the best α-glucosidase producer strain

Microbial identification was performed via morphological,

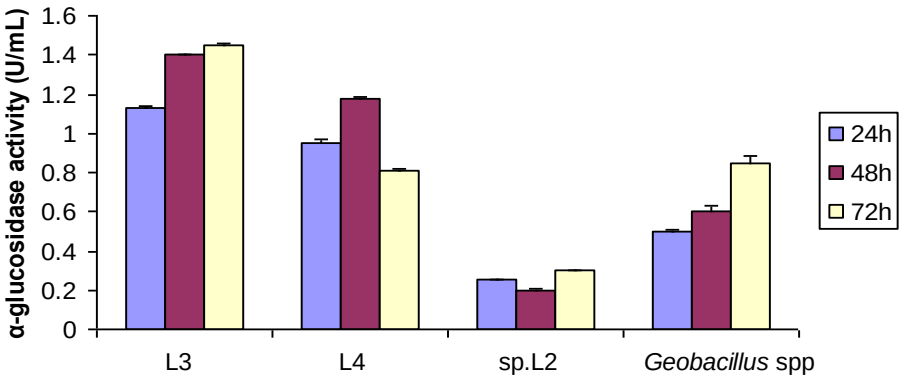

Figure 1. α-Glucosidase activity of producer strains at different incubation times (24, 48 and 72 h). The samples were incubated at 50°C (L3 and L4), 60°C (*Geobacillus* spp.) and 70°C (L2).

biochemical studies and 16S rRNA gene sequence analysis. The pure bacterial strain was streaked on NA agar and incubated overnight at 37°C. A single colony was subjected to Gram staining and observed under light microscope. Genomic extraction was then carried out using DNeasy Kit (Qiagen, Germany) according to the manufacturer's instruction. A set of universal primers; F (5'-GAG TTT GAT CCT GGC TCA G-3') and -R (5'-CGG CTA CCT TGT TAC GAC TT-3') was used to amplify 16S rRNA gene. The steps and conditions used for PCR cycle (30 cycle), were pre-denaturation -94°C for 4 min, denaturation- 94°C for 1 min, annealing -55°C for 1 min, extension -72°C for 1 min, final extension -72°C for 1 min. The PCR product was then sequenced using an ABI PRISM 377 DNA sequencer (Applied Biosystems, USA). In the phylogenetic analysis, homology search was carried out using the basic BLASTN search program at the NCBI website. A neighbor-joining phylogenetic tree was constructed with the MEGA package version 5 (Tamura et al., 2004).

Isolation of the α-glucosidase encoding gene

A set of degenerate primer, F (5'-GAR TTY GGN ACN ATG GAY GAY TTY G-3') and R (5'- AAN CCR TCD ATN CCY TTR TCN ARC CAC C -3') that was targeted on the α-glucosidase highly conserved regions from a variety of microorganisms were used to amplify partial α-glucosidase gene from the genomic DNA of the identified bacterium. DNA was amplified by PCR as follows: pre-denaturation at 94°C for 4 min, and then 30 cycles of denaturation at 94°C for 1 min, annealing at 60.5°C for 1 min, extension at 72°C for 1 min. A new set of primer; F (5'- TTG AAA AAA ACA TGG TGG AAA GAG-3') and R(5'- TTA TTC TTT CCA GAT GTA TAC GC -3') was designed to amplify the full α-glucosidase gene using similar PCR conditions as above, with a slight modification in the annealing temperature that was set at 56°C. Gene analysis using multiple sequence alignment and phylogenetic tree was carried out using MEGA 5 software.

Cloning and expression into *E. coli*

The amplified α-glucosidase gene was cloned using pBAD and pTrcHis @ TOPO expression vectors (Invitrogen®) and transformed into *E. coli* Top 10. The transformation mixture was grown overnight on α-MUG-LB-starch screening agar plate supplemented with 100 µg/ml ampicillin for direct screening of positive transformants. Isopropyl-β-D-thio-galactoside (IPTG) was used as inducer for pTrcHis2 system, while L-arabinose as inducer for pBAD system. The positive transformants of both systems were streaked onto LB plate containing 100 µg/ml ampicillin and grown overnight at 37°C. A pure single colony was then inoculated into 2 ml LB broth supplemented with ampicillin and incubated at 37°C overnight under shaking (250 rpm) conditions until the absorbance reading (A_{600}nm) reached 1-2. The overnight culture (0.1 ml) was then transferred into fresh 10 ml of LB containing ampicillin (100 µg/ml) until the absorbance reading (A_{600}nm) reached ~0.5, and induced with 0.02% (w/v) L-Arabinose for pBAD system and 1 mM IPTG for pTrcHis2 system. Culture (10 ml) was harvested and centrifuged at 10,000 rpm for 10 min. The pellet was then resuspended with 2 ml of potassium phosphate buffer (pH 7.0) and then sonicated with Branson sonifier 250 (output: 2, duty cycle: 30 and 2 min). The cell lysate was assayed colorimetrically as described previously followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis test (SDS-PAGE).

RESULTS

Isolation and screening of putative α-glucosidase producing bacteria

Five isolates from water samples (SR 38, SR 40, SR 74, L2 and *Geobacillus* spp.) from Slim River, Perak were successfully grown at 50-70°C while the samples from Telaga Air Hangat, Langkawi (L3 and L4) at 55°C. After qualitative screening with 4-methylumbelliferyl-α-D glucoside (α-MUG) plate overlay method, 4 out of 7 samples (L2, L3, L4 and *Geobacillus* spp.) gave positive putative α-glucosidase production, because these microorganisms exhibited fluorescence around the colonies, meanings hydrolysis of α-MUG by α-glucosidase.

All positives α-glucosidase producers were inoculated separately. L3 had been identified as the best α-glucosidase producer as its enzyme activity was 1.47 U/ml followed by L4, 1.18 U/ml, L2, 0.3 U/ml and *Geobacillus* spp., 0.9 U/ml (Figure 1). PM1 was the best

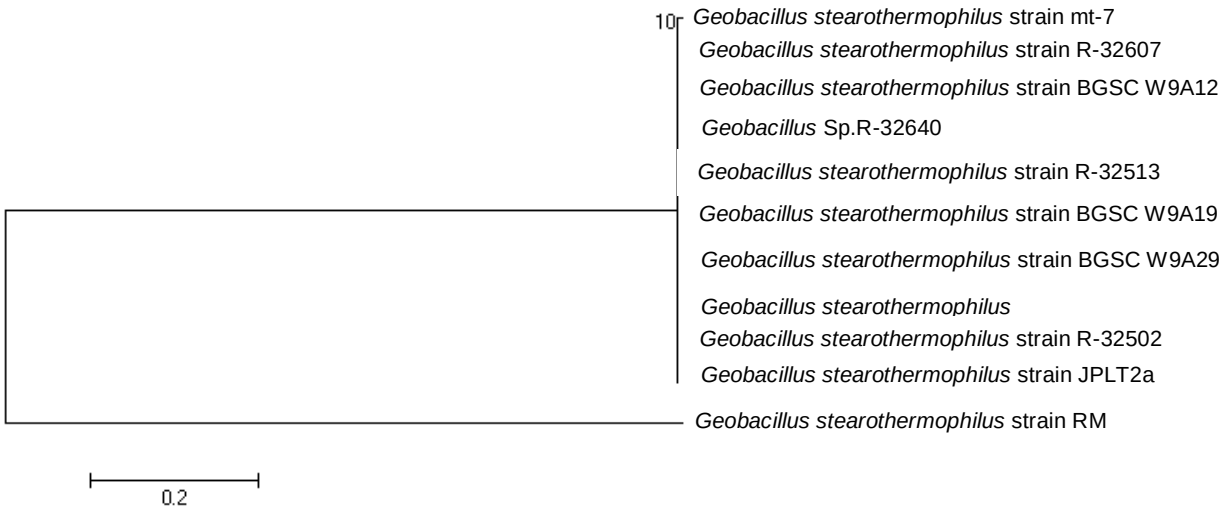

Figure 2. Neighbour-joining evolutionary distance phylogenetic tree based on the 16S rRNA gene sequences of strain RM and the representative members of genus *Geobacillus* of the family *'Bacillaceae'*. Bootstrap values (%) are based on 500 replicates and shown for branches with more than 50 % bootstrap support. Bar indicates 0.2 substitutions per nucleotide position.

medium for α-glucosidase production in *Geobacillus* spp. and L2 while PM2 for L3 and L4 with its growth temperature as stated above.

Identification of isolate L3 as *G. stearothermophilus* strain RM

Isolate L3 that gave the highest α-glucosidase activity, was identified and further studied. Morphological and biochemical studies showed that the bacterium exhibited rod shape and cream when incubated on NA (1% agar) at 60°C with a width and length of 0.9 - 1.1 µm and 3.0 - 5.0 µm respectively. Gram staining indicated that this bacterium was gram positive with oval spore's formation and positive in catalase activity. In addition to it's facultatively anerobic growth, the analysis of the cellular fatty acids also showed a good correspondence to the profile of the typical thermophilic *Bacillus* strains. A test on the formation of acetylmethylcarbinol, known as the Voges-Proskauer (VP) test was also carried out and the output was no reaction. Hydrolysis tests on four different substrates (starch, gelatin, casein and Tween 80) showed that this isolate preferably hydrolyzed starch and amylase activity were positive. Its optimum temperature was at 55°C and best grown at pH 6.8 with the ability to utilized a wide range of carbon sources including D-glucose, L-Arabinose, D-Fructose, D-Raffinose, D-Lactose, L-Arabinose and D-Mannose acids.

The partial 16S rRNA gene sequence revealed that this strain RM was 1.5 kb nucleotides long. Phylogenetic tree of 16S rRNA was constructed using the neighbour-joining method and analyzed using MEGA 5 to show the evolutionary relationships of 11 taxa including the studied sequence, *G. stearothermophilus* strain RM through sequence alignment with CLUSTALW (Figure 2). It showed the position of strain RM among the species of genus *Bacillus* and *Geobacillus* which fell into the phylogenetic group 5 of endospore-forming bacteria. According to the 16S rRNA gene sequence similarity, the strain was found to be closely related to *G. stearothermophilus* with 99% similarity. In addition, from the alignments, around 350 nucleotides of these aligned sequences have conserved region at around nucleotide 1163 to 1473. The GenBank accession number for this deposited sequence is GU045559 as *G. stearothermophilus* strain RM.

Cloning and expression of α-glucosidase gene in *E. coli*

The degenerate primer for α-glucosidase gene that had been designed successfully amplified around 300 bp conserved region of the α-glucosidase gene from this isolate L3. This degenerate primer region had been designed on the basis of all the bacterial α-glucosidases would have this conserved amino acids region ((EFGTMD(E)DF and WWLD(E)KGIDGF)) as reported in *G. strearothermophilus*, *B. subtilis* (Z99120-1), *Bacillus halodurans* C-125, *Staphylococcus aureus* (AP004827-121) and *Clostridium perfringens* (Hung et al., 2005). The BLAST result obtained from this amplified PCR product showed that the gene was 98% similar with *Geobacillus sp.* HTA-462 gsj gene for α-glucosidase, complete coding sequences. From this reference sequences, a new set of primer was designed and successfully amplified the complete sequence of α-glucosidase gene (Figure 3).

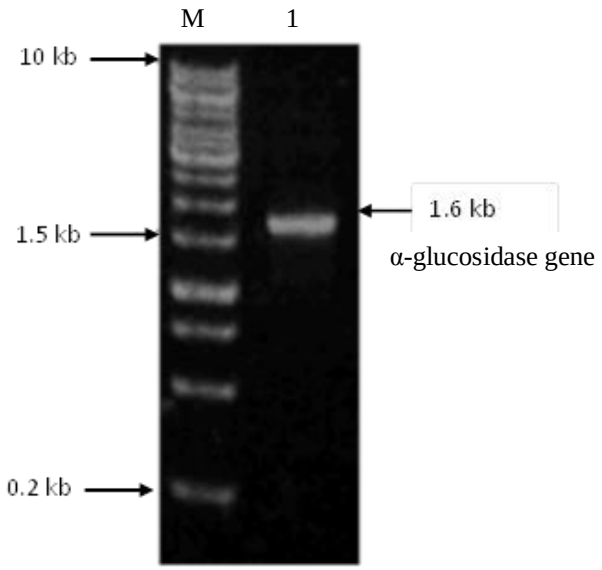

Figure 3. Purified PCR product of the complete α-glucosidase gene.

The PCR product was then sequenced and the BLAST result showed that the gene was 99% similar to *Geobacillus* sp. HTA-462 gsj gene. The open reading frame (ORF) composed of 1,668 bp encoding a protein of 555 amino acids with a predicted molecular mass of 65.173 kDa (Figure 4). There was a putative ribosome-binding sequence, 5′-AAAGGGGG-3′, located 8 bp upstream of the TTG start codon. The ORF was terminated with a TAA stop codon.

Gene analysis using multiple sequence alignment and phylogenetic tree between the studied α-glucosidase and other α-glucosidases showed that the gene had quite a high evolutionary history distance with the sum of branch length = 19.37209170 (Figure 5). It was also shown that this gene had high similarity to α-amylase. As shown in Table 1, analysis based on G+C content of α-glucosidase from different families of α-glucosidase, covering a few genera was compared as a preliminary determination of evolutionary relationship. The G+C content of α-glucosidase from *Desulfurococcus fermentans* gave the highest G+C content (56.8%) followed by the studied α-glucosidase and *Geobacillus sp*. HTA-462 gsj (52.1%). As for these 4 strains, *Geobacillus sp*. C56-T3, *Geobacillus kaustophilus* HTA-426, *Geobacillus* sp. Y412MC61 and *G. stearothermophilus* ATCC (D84648.1), the G+C content was approximately 51% and the lowest G+C content was α-glucosidase from *Geobacillus thermodenitrificans.* (Table 2)

The full length of α-glucosidase gene was amplified in order to clone and express the gene in *E. coli* system. The recombinant clones that exhibited fluorescence on α-MUG-LB-starch screening agar plate supplemented with100 µg/ml ampicilin were chosen for further analysis (Figure 6). Sequencing result of the recombinant plasmids revealed that the recombinant plasmids (pBAD/α-glu) or (pTrcHis2/α-glu) harboured an α-glucosidase gene of 1668 bp (Figure 7). The amino acids encoded by α-glucosidase gene are 536 amino acids with deduced molecular mass and pI of 62.776 kDa and 5.56, respectively. SignalP V3.0 analysis revealed that this gene has no signal peptide. After inducing the positive recombinant *E. coli* (pBAD and pTrcHis2-TOPO harbouring α-glucosidase gene) for 12 h using 0.2 % of L-arabinose and 1 mM IPTG respectively, the assay showed that expression level increased sharply as compared to wild type at 12 h to about 5-folds (5.13 U/ml) in pBAD while 3-folds (3.6 U/ml) in pTrcHis2 (Figure 8).

DISCUSSION

There are many types of amylases, for example, α-amylase, ß-amylase and α-glucosidase. α-Amylase reacts at the initial stage of starch hydrolysis and gives maltose or glucose as a product, while α-glucosidase hydrolyses maltose into glucose. Thermostable α-glucosidases have a number of potential applications due to their wide range of substrate specificity and their ability of catalyzing transglucosylation reactions (Cihan et al., 2010). In this study, temperature and production media were studied to facilitate α-glucosidase production. These optimal temperatures (L2 at 70°C, *Geobacillus* spp. at 60°C, while *G. stearothermophilus* strain RM and L4 at 55°C) were due to their original temperatures where the samples had been isolated. Telaga Air Hangat, Langkawi and Slim River, Malaysia were chosen as the screening location since they are well known as hot spring location harboring lowest and highest thermophiles temperature range respectively (40-100°C). PM1 was the best production media for *Geobacillus* spp. and L2, while PM2 for *G. stearothermophilus* strain RM and L4. Both media contained starch as a carbon source which had proven to enhance the production of α-glucosidase. In a study from (Alpana et al., 1980), carbon sources such as maltose and starch also favored good amylase synthesis with starch being most favorable in this respect.

16S ribosomal RNA (rRNA)-based identification of bacteria potentially offers a useful alternative when phenotypic characterization methods fail. The 16S rRNA gene are highly conserved among prokaryotes and found to amplify the whole region of the rRNA gene which is 1500 bp. The α-glucosidase gene had been successfully amplified and the amino acids sequence of α-glucosidase from this *G. stearothermophilus* strain RM shows the same conserved region as the other reported wild type bacteria as stated previously (Shimizu et al., 2002; Takami et al., 2000; Takii et al., 1996).

However, as reported previously, this gene had no signal peptide hence make this protein intracellular

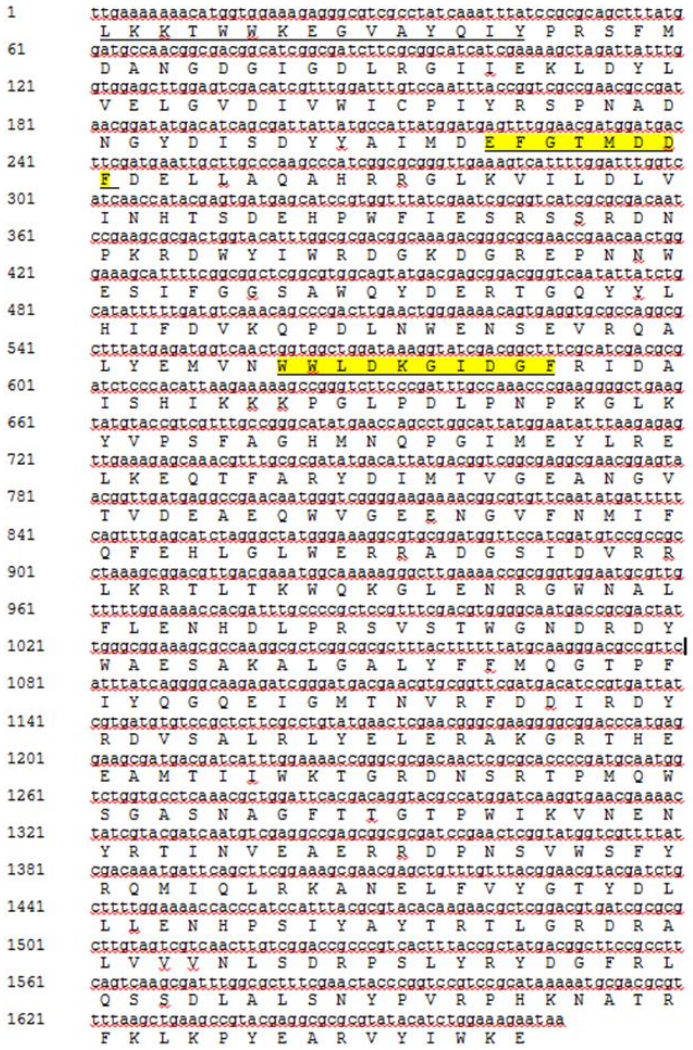

Figure 4. The DNA sequence of thermostable α-glucosidase gene from *G. stearothermophilus* strain RM. Note: N terminal sequence (underlined) and two conserved sequences (shaded).

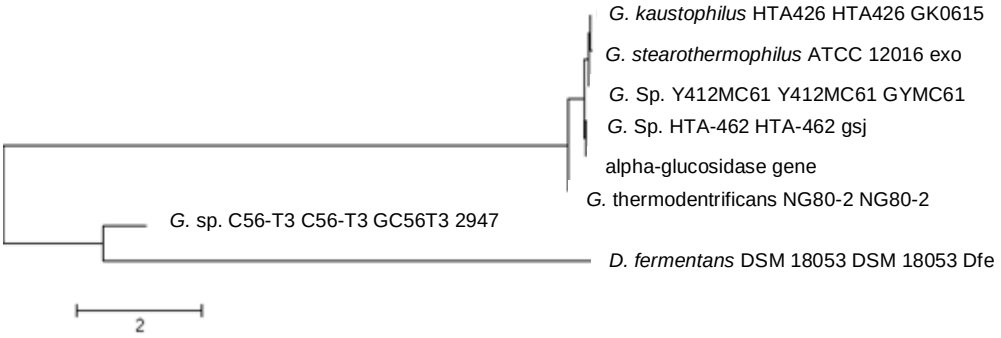

Figure 5. Phylogenetic position of the studied α-glucosidase gene with other α- glucosidase from *G. stearothermophilus*, *G. kaustophilus*, *Geobacillus* sp., *G. thermodenitrificans* and *Desulfurococcus fermentans.* Phylogenetic tree was inferred by using the neighbour-joining methods. The software package MEGA 5 was used for analysis. The scale bar indicates 2 substitutions per nucleotide position.

Table 1. Morphological and biochemical studies of *G. stearothermophilus* strain RM.

Biochemical	Morphological
1. Reaction with acetylmethylcarbinol, known as the Voges-Proskauer (VP) test was also carried out and the output was no reaction.	1. Rod shape with a width and length of 0.9-1.1 µm and 3.0-5.0 µm respectively
2. Hydrolyzed starch	2. Gram positive with oval spore's formation
3. Positive in catalase activity	3. Facultative anaerobic
4. Ability to utilized D-glucose, L-Arabinose, D-Fructose, D-Raffinose, D-Lactose, L-Arabinose and D-Mannose acids	4. Optimum temperature at 55°C and best grown at pH 6.8

Table 2. G+C content comparison among different strains of α-glucosidase producer.

α-glucosidase	G+C content (%)
Geobacillus sp. HTA-462 gsj (AB154818.1)	52.1
Geobacillus sp.C56-T3 (CP002050.1)	51.7
Geobacillus kaustophilus HTA-426 (BA000043.1)	51.4
Geobacillus sp. Y412MC61 (CP001794.1)	51.6
Geobacillus stearothermophilus ATCC (D84648.1)	51.6
Geobacillus thermodenitrificans (CP000557.1)	47.5
Desulfurococcus fermentans (CP001619.1)	56.8
Studied α-glucosidase gene	52.1

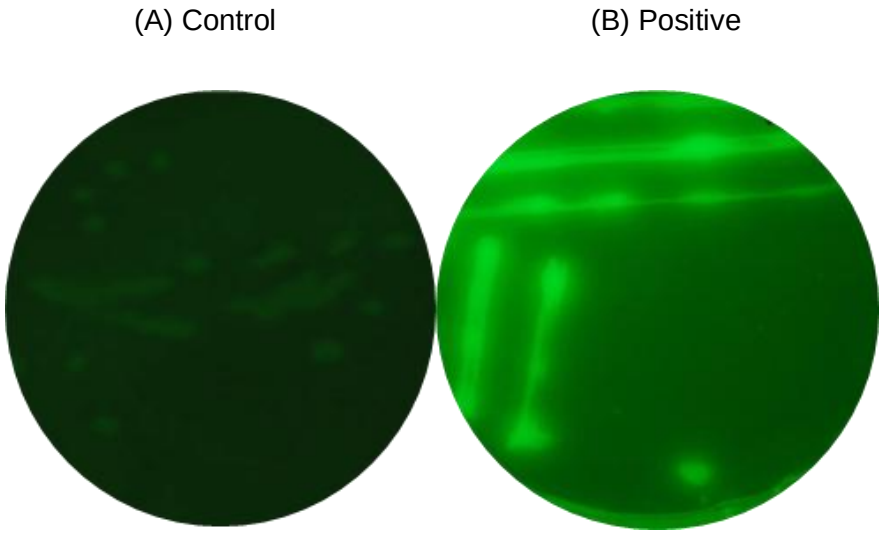

Figure 6. α-MUG-LB agar plate showing control and positive α-glucosidase recombinant colonies.

protein compared to other reported *Geobacillus* proteins which were usually extracellular (Davis et al., 2006). As reported by Cihan et al. (2010), α-glucosidase is usually occurring in microorganisms as intracellular, extracellular, or cell-bound enzymes. The extracellular α-glucosidase activities of *B. thermoamyloliqefaciens* KP1071 (Suzuki et al., 1992), *G. thermodenitrificans* HR010 (Ezeji et al., 2005) and *Geobacillus* sp. A333 were detected outside of the cell after the late exponential growth phase as in the case of *G. thermodenitrificans* F84a. The significance of studying the G+C content of a gene was due to the stability of GC rich gene hence concluding that α-glucosidase with high G+C content might represent a more stable enzyme compared to the one with low G+C

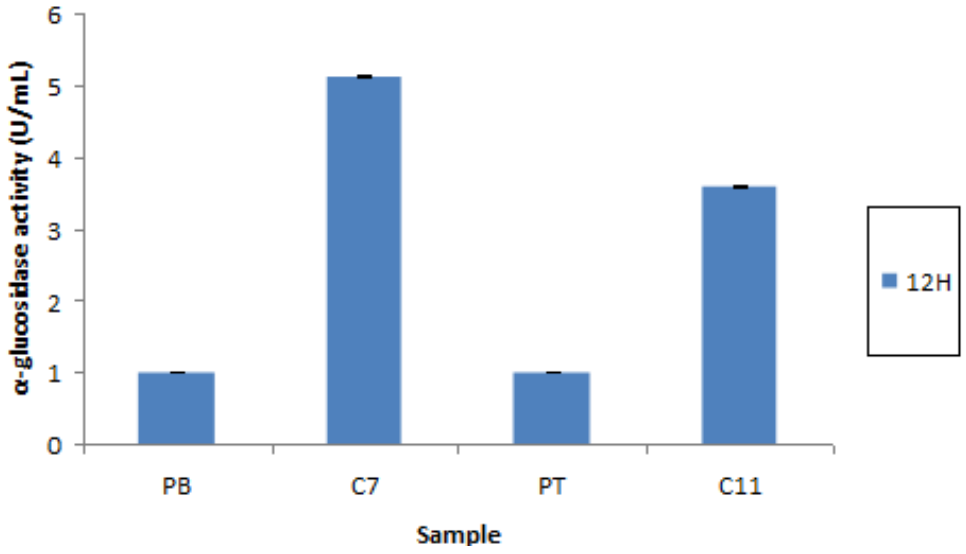

Figure 7. Expression of α-glucosidase gene by *E. coli* Top 10 cultures harbouring recombinant plasmid pBAD/ α-glucosidase and pTrcHis2/ α-glucosidase at 55°C. The cultures was induced with 0.2 % L-arabinose at A600nm ~ 0.5 up for 12 h. Note: Ctrl PB- empty pBAD vector as a control, C7- recombinant plasmid pBAD/ α-glucosidase, Ctrl PT- empty pTrcHis2 vector as a control, C11- recombinant plasmid pTrcHis2/ α-glucosidase. The test was carried out in 3 replications at 55°C.

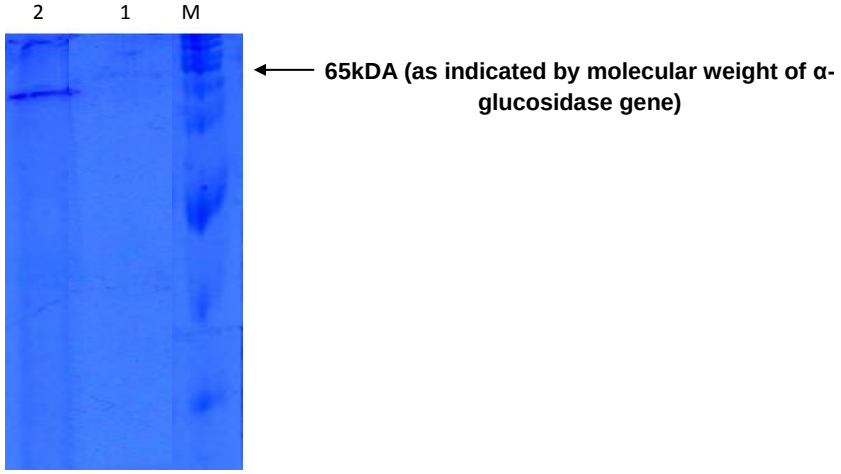

Figure 8. SDS-PAGE (12%) of expressed α-glucosidase after induced for 12 h. M: standard protein markers. Lane 1: control expression; Lane 2: recombinant culture *E. coli* Top 10 (pBAD/ α-glucosidase).

content. It is known that the GC content of genes strongly influences the resulting proteins' hydrophobicity, which is the main determinant of folding stability. Proteins that are too hydrophylic face unfolding problems and proteins that are too hydrophobic face misfolding and aggregation problems (Mendez et al., 2010).

TOPO-TA expression vectors (pBAD and pTrcHis2) were chosen as they were efficient in expressing heterologous protein. Comparison between the two vectors in term of expression level was made and it was seen clearly that pBAD vector gave higher yield compared to pTrcHis2. The difference between these two vectors was the promoter driven the expression. As for pBAD, expression of the gene was controlled by the AraC activator. When a gene is cloned behind the araBAD promoter, the AraC, which a gene product encoded on the pBAD plasmid positively regulates this promoter. Heterologous gene is induced to high levels on media containing arabinose. Moreover, expression from pBAD is tightly shut off on media containing glucose but lacking

arabinose. In addition, the tight regulation of pBAD by AraC is useful for expression of potentially toxic or essential genes and optimizes protein solubility (Guzman et al., 1992).

The increment of the activities from wild type to recombinant α-glucosidase was compared with a study by Aneta et al. (1993), which their activity of recombinant α-glucosidase increased to 1.3-fold higher compared to wild type, as in this study, the increment was 5-folds. As mentioned previously, this enzyme usually hydrolyses glycosidic bonds, however, it is also able to catalyze the stereospecific formation of such linkages (Scigelova et al., 1999). It was due to the transglycosylation potential of this enzyme. This transglycosylation affects the hydrolysis activity in which the end product is modified, for example, it conjugates sugars to biologically relevant materials improving their chemical properties and physiological functions (Kren and Martinkova, 2001). Among the α-glucosidases of thermophilic and endospore-forming bacteria, only some were stated as α-glucosidases catalyzing transglycosylation reactions. α-glucosidases owing high transglycosylation activities were reported as *Bacillus* sp. SAM1606 (Nakao et al. 1994), *Geobacillus* sp. HTA-462 (Hung et al., 2005), and *Geobacillus* sp. A333 (Cihan et al., 2010). In a biotechnological point of view (food industry, production of glycoconjugates, etc.) this ability of the enzyme is good, but in the case of focusing at one end product (glucose), it's kind of affecting the enzyme activity and lower the amount of desired end product.

In conclusion, a new *Geobacillus* species from hotspring in Malaysia which is capable of producing an intracellular thermostable α-glucosidase has been revealed. Since there are many reports on α-glucosidase production mainly from eukaryotic sources, such as yeast and fungi, this study on bacterial α-glucosidase seems important in addition to the existing knowledge. In addition, this α-glucosidase protein was found to be stable from its molecular study. Prokaryotic system is easy to manipulate, easy to obtain and deal, making this study relevant for further development. The new bacterial source for this gene was successfully identified with some optimization on its growth temperature and production media undertaken.

ACKNOWLEDGEMENT

The authors would like to acknowledge MOSTI, Malaysia (MGI) for financial support through Science Fund (No.07-05-MGI-GMB003).

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