Jurnal Teknologi

Full Paper

CHARACTERIZATION OF THERMOSTABLE BETA-1,4-GALACTANASE AND ITS APPLICATION IN HYDROLYSIS OF PECTIN FROM SWEET POTATO (*IPOMOEA BATATAS* (L.) LAM) PEELS

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Article history
Received
21 June 2021
Received in revised form
12 July 2021
Accepted
26 July 2021
Published online
20 August 2021

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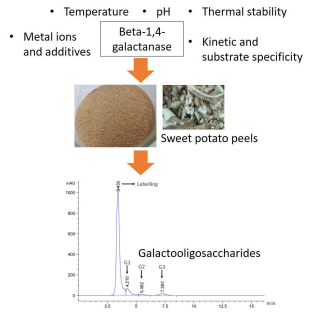
Graphical abstract



Abstract

Galactooligosaccharides (GOS) synthesis has received much attention due to its prebiotic function. Beta-1,4-galactanase responsible for the hydrolysis of galactan plays an important role in producing GOS from biodegradation of this pectin component. In this study, beta-1,4-galactanase (BgcGC) from a thermophilic *Geobacillus mahadii* Geo-05 was heterologously expressed in *Escherichia coli* (*E. coli*) and characterized. The optimum temperature of BgcGC was at 60°C and stable from 20-60°C while optimum pH was at 6 and stable from pH 4-10. BgcGC showed high catalytic efficiency towards potato galactan (873.8 ml mg^{-1} s^{-1}) and lupin galactan (1694.4 ml mg^{-1} s^{-1}). The activity of BgcGC was not significantly affected with the presence of 100 mM K^+, Tween-20 and 2-mercaptoethanol. Application of BgcGC towards pectin-containing galactan oligomer extracted from sweet potato peels resulted in galactose and GOS synthesis as revealed by high performance liquid chromatography analysis. Thus, this enzyme has a potential to be one of the enzyme candidates involves in pectin complex degradation to produce GOS.

Keywords: Beta-1,4-galactanase, thermophilic, pectin, galactan, galactooligosaccharide

Abstrak

Sintesis galaktooligosakarida (GOS) telah mendapat banyak perhatian disebabkan fungsi prebiotiknya. Beta-1,4-galaktanase bertanggungjawab terhadap hidrolisis galaktan memainkan peranan penting dalam penghasilan GOS daripada biodegradasi komponen pektin ini. Di dalam kajian ini, beta-1,4-galaktanase (BgcGC) daripada *Geobacillus mahadii* Geo-05 termofilik telah diekspres secara heterologi di dalam *Escherichia coli* (*E. coli*) dan dicirikan. Suhu optima BgcGC adalah pada 60°C dan stabil antara 20-60°C manakala pH optima ialah pada pH 6 dan stabil antara pH 4-10. BgcGC menunjukkan keefisienan pemangkin yang tinggi terhadap galaktan kentang (873.8 ml mg^{-1} s^{-1}) dan galaktan lupin (1694.4 ml mg^{-1} s^{-1}). Aktiviti BgcGC tidak begitu terkesan dengan kehadiran 100 mM K^+, Tween-20 dan 2-merkaptoetanol. Aplikasi BgcGC terhadap pektin yang mengandungi polimer galaktan ekstraksi daripada kulit ubi keledek menghasilkan galaktan dan GOS sebagai produk hidrolisis seperti yang ditunjukkan oleh analisis kromatografi cecair berprestasi tinggi. Oleh itu, enzim ini berpotensi untuk menjadi salah satu calon enzim yang terlibat dalam degradasi kompleks pektin untuk menghasilkan GOS.

Kata kunci: Beta-1,4-galaktanase, termofilik, pektin, galaktan, galaktooligosakarida

1.0 INTRODUCTION

Carbohydrate is one of the most important biomolecules commonly found in nature. It serves as energy storage, metabolic intermediates and becoming a structural element in the living cells. To keep its function, an enzyme or a catalyst is important to accelerate the reaction or mechanism involved. Hydrolysis of carbohydrate assisted by a group of the enzyme is known as glycosyl hydrolase (GH).

GH also called glycosidase (EC 3.2.1.-), is a widespread group of enzymes responsible for the cleavage of glycosidic bond in carbohydrate molecule. Its prominent application in degrading biomass components such as cellulose, hemicellulose, pectin and starch make it an outstanding class of enzyme to be studied. A dedicated database for the carbohydrate enzyme named as Carbohydrate-Active Enzymes database (CAZy; http://www.cazy.org) has been established to gather information regarding the most influential enzymes in the universe [1]. GH are classified into families according to their amino acid sequence similarities which reflect the protein folding. The vast applications of glycosidases ranging from the harnessing of plant biomass degradation to their roles in human physiology and health, has triggered a large scientific group with an interest in glycosidase chemistry.

Beta-1,4-galactanase (EC 3.2.1.89) is a GH under family 53 which catalyze hydrolysis reaction of beta-1,4-galactan in an endo-mode action. It has been applied in pectic polysaccharide degradation and mechanism of action [2, 3]. The action of beta-1,4-galactanase together with other enzymes has been proven to facilitate the hydrolysis of complex plant biomass [4, 5]. To fulfill the industrial need, the enzyme property to withstand high temperature gives an advantage in reducing the chance for microbial growth and improving solubility of substrate at high concentration.

Hydrolysis reaction of beta-1,4-galactanase resulted in the galactooligosaccharide (GOS) synthesis. GOS has been widely used in human milk formula due to its prebiotic role and it is mainly obtained from transglycosylation reaction of beta-galactosidase towards lactose [6, 7, 8]. However, in recent years, galactan from pectin extract has been studied for the GOS synthesis [4]. GOS produced from galactan has been proven to possess prebiotic effect on the human gut microflora such as *Bacteroides thetaiotaomicron*, *Lactobacillus* and *Bifidobacterium* sp. [3, 9, 10].

Galactan can be found in a heteropolysaccharide structure of pectin which comprises of three main regions known as homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II [11, 12]. Meanwhile, pectin polysaccharides are abundantly found in primary plant cell wall of dicotyledon which makes it becomes the emerging source of galactan that is being explored as an alternative substrate to lactose for GOS synthesis.

Depolymerisation of pectin requires physical, chemical or enzymatic process [13]. Enzyme reaction provides mild conditions and usually safer than chemical reaction, thus become favorable in biodegradation of biomass like pectin. The incorporation of thermostable enzyme may be essential as pectin operates at high temperature due to its viscosity property [14]. This study described the biochemical characterization of beta-1,4-galactanase and its potential application to be used in hydrolysis of pectin from sweet potato (*Ipomoea batatas* (L.) Lam) peels to produce GOS.

2.0 METHODOLOGY

2.1 Bacterial Strains, Plasmid and Chemicals

Beta-1,4-galactanase gene from *Geobacillus mahadii* Geo-05 (GenBank accession number: ARB18040.1) was synthesized with codon-optimized sequence by GenScript USA Inc and cloned into pCold-I (Takara, USA) as the expression vector. The constructed plasmid was named as BgcGC and transformed into *E. coli* JM109 and *E. coli* BL21 (DE3) (Promega, USA) as the cloning and expression host, respectively. The transformed cells were grown in Luria Bertani medium supplemented with 100 µg/ml ampicillin and incubated for 16-18 hours with 200 rpm shaking at 37°C for growth. Chemicals used in this study were either analytical or HPLC grade from Sigma Aldrich (Missouri, USA), Merck (Darmstadt, Germany) and Thermo Fisher Scientific (Massachusetts, USA).

2.2 Enzyme Expression and Purification

Expression of BgcGC was modulated as per mentioned in the plasmid manual employed in this study. BgcGC was induced with 0.5 mM isopropyl beta-D-1-thiogalactopyranoside (IPTG) when OD_{600} reached ~0.5 and switched to 20°C incubation for 8 hours. An 800 ml of cell culture were harvested by centrifugation at 3,220 × *g*, 30 min at 4°C using 250 ml conical bottle in swing bucket operated by Eppendorf Centrifuge 5810 R (Hamburg, Germany). The cell pellets were suspended in 5% (v/v) of binding buffer (150 mM Tris, 25 mM NaCl, 20 mM 2-mercaptoethanol and 20% glycerol, pH 8) from the volume of culture followed by sonication in an ice bath. A 0.5 mM phenylmethanesulfonyl fluoride (PMSF) was added to the suspension before and after sonicated to avoid protein degradation by proteases. The cell debris was removed by centrifugation at 9509 × *g* for 30 min at 4°C.

The supernatant constituting the enzyme crude extract was loaded into a self-packed Ni Sepharose 6 Fast Flow (GE Healthcare, UK) column. Purification

procedure was guided by the manufacturer's manual of resin used in this study [15]. Binding buffer was applied to the column before elution buffer (150 mM Tris, 25 mM NaCl and 300 mM imidazole, pH 8) was loaded into the column to recover the Ni-binding protein.

Second step purification was done using HiTrap Q HP, 5 ml (GE Healthcare, UK) with an ÄKTAPrime Plus Purification System (GE Healthcare, UK). L-histidine buffer (20 mM L-histidine, pH 6) was applied to the column while 20 mM L-histidine buffer containing 1 M NaCl (pH 6) was used to elute the protein using gradient mode. All the procedures described were performed at 4°C.

2.3 Beta-1,4-galactanase and Protein Assay

Beta-1,4-galactanase assay procedure using 4-hydroxybenzoic acid hydrazide (PAHBAH) reagent was adapted from the previous studies [10, 16]. The hydrolysis activity of BgcGC was measured by incubating 50 µl of diluted enzyme in 125 µl of 1% (w/v) potato galactan from Megazyme (Bray, Ireland) in citrate-phosphate buffer, pH 6.0 (Mlilvaine buffer, 0.1 M citric acid and 0.2 M disodium hydrogen phosphate). The enzyme mixture was then incubated at 60°C for 30 min. A 5 µl of the reaction mixture was taken out and added into 1.5 ml of PAHBAH reagent and boiled for 5 min to stop the reaction. The absorbance was read at 410 nm. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 µ mol of galactose per min under experimental conditions. The protein concentration was measured by the Bradford method with bovine serum albumin as a standard [17].

2.4 Biochemical Characterization, Substrate Specificity and Enzyme Kinetics

The optimum temperature for the purified BgcGC was measured by conducting assay at different temperature ranging from 20 to 90°C. The optimum pH was determined by conducting assay at 60°C using appropriate buffers. The buffers used were citrate phosphate (McIlvaine buffer) for pH 2.2–6, sodium phosphate buffer (pH 6–8) and carbonate-bicarbonate buffer (pH 9–10). The enzyme activity was calculated as a relative activity using the optimum condition as a reference of 100% activity. Thermostability of the purified BgcGC was evaluated by pre-incubating the enzyme at different temperature (20-70°C) for 30 min prior to enzymatic assay. The pH stability was investigated by pre-incubating the enzyme at 4°C in buffer with different pH value (2–10) for 30 min prior to enzymatic assay. The residual activity for both parameters were determined under optimal assay conditions with the enzyme activity before pre-incubation regarded as 100%.

The thermal inactivation of BgcGC was analysed by assaying the residual activity at the specific intervals after incubating the enzyme at different temperature (20–70°C). ln [residual activity] versus time was plotted according to the first-order kinetics [18]. Deactivation rate constant (K_d) of irreversible thermal denaturation was obtained from the slope of the plot and used to calculate the half-life of thermal inactivation ($T_{1/2}$), represented by ln_2/K_d.

The effect of metal ions and additives on the enzyme activity were examined by adding the reagents to the assay reaction mixture at concentrations of 5, 50 and 100 mM for metal ions and 1% (v/v) for additives. Enzyme activity was expressed as relative values (%) with reference to the activity of the enzyme without metal ion or additive.

The substrate specificity was determined using potato galactan, lupin galactan, debranched arabinan, rhamnogalacturonan and pectin from apples as substrates. Michaelis-Menten equation was applied to calculate kinetic parameters (V_{max}, K_m, K_{cat} and K_{cat}/K_m) using Lineweaver-Burk plot.

2.5 Extraction of Pectin from Sweet Potato Peels (SPP) and Monosaccharide Analysis

Extraction of pectin from SPP was prepared as described previously with minor modifications [19]. Monosaccharides composition of the SPP pectin was identified using a modified method previous studies [20, 21]. A 2.5 mg SPP pectin was dissolved in 500 µl of 2 M trifluoroacetic acid. The mixture was incubated at 95°C in a thermoblock (Thermomixer comfort; Eppendorf) for four hours for the hydrolysis to occur. Then, the remaining acid was dried in 70 – 80°C oven for overnight. The dried samples was re-dissolved in distilled water and filtered using Vivaspin 20, 30,000 MWCO PES (Sartorius Stedim Biotech GmbH, Germany) to remove peptides and proteins. The samples were filtered using Vivaspin 20, 0.2 µm PES (Sartorius Stedim Biotech GmbH, Germany) prior to analysis using Bio-Rad Aminex HPX-87H (300 mm × 7.8 mm; Bio-Rad, USA) column attached to a High Performance Liquid Chromatography (HPLC) (Agilent Infinity 1260, USA) equipped with refractive index (RI) detector. Rhamnose (Rha), galactose (Gal), galacturonic acid (GalA) and arabinose (Ara) were used as standard.

2.6 Hydrolysis of SPP Pectin with BgcGC

A 900 µl of 10 mg/ml SPP pectin dissolved in 50 mM sodium acetate buffer pH 5.5 was added with 100 µl endo-polygalacturonanase M2 (endo-PG) from *Aspergillus aculeatus* (Megazyme, Bray, Ireland) at a concentration of 40 units/g pectin, following procedure adapted from Khodaei & Karboune (2013). The reaction was carried out at 35°C and 300 rpm for 20 hrs in thermoblock before it was stopped by boiling for 5 min. Then, 125 µl lof the stopped reaction was added with BgcGC in accordance to beta-1,4-galactanase assay.

Table 1 Purification table of two-step BgcGC purification scheme in *E. coli*

Step	Volume (ml)	Enzyme activity (U/ml)	Total Activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	20	13.0	259.9	90.7	2.9	1.0	100.0
Ni-NTA	5	30.5	152.6	18.4	8.3	2.9	58.7
Anion exchange	3	17.6	52.9	1.7	31.8	11.0	20.4

2.7 Galactooligosaccharide analysis using HPLC-UV

A 10 µl of sugar sample was added into 40 µl labeling reagent (90 mg/ml 4-aminobenzoic acid methyl ester (ABEE) and 9 mg/ml sodium cyanoborohydrate dissolved in methanol:acetic acid;9:1). The mixture was heated in 85°C thermoblock for 45 min in a tightly closed container before 0.5 ml water was added to the reaction mixture. Then, a 0.75 ml diethyl ether was added, mixed and pipetted out to remove excess of 4-ABEE labeling. The washing step was repeated five times. The aqueous layer was lyophilized, and the residue was dissolved in a 100 µl water and 100 µl acetonitrile. The ABEE-derivatized sugars were filtered using 0.2 mm-pore-sized syringe filter prior to injection into the HPLC system (Agilent Infinity 1260, USA) equipped with UV-Vis detector and an automatic injector. An XBridge™ Amide 5 µm (4.6 × 250 mm; Waters, USA) column and the Guard Cartridge were employed for the sugar quantification. The analysis was monitored at 304 nm and the column was operated at 28°C under the gradient mode. The mobile phase used were Buffer A (20% of 100 mM ammonium acetate buffer, pH 3.85, 80% acetonitrile) and Buffer B (20% of 100 mM ammonium acetate buffer, pH 3.85, 20% acetonitrile).

3.0 RESULTS AND DISCUSSION

3.1 Sequence analysis of BgcGC

A 2,355 nucleotides sequence encodes for a mature 785 amino acid (without its native 87 base pair signal peptide, predicted by SignalP server; http://www.cbs.dtu.dk/services/SignalP/) of beta-1,4-galactanase gene from a thermophilic *G. mahadii* Geo-05 (GenBank: KY744702.1) was expressed, purified and characterized with codon optimization to achieve optimum expression of BgcGC in *E. coli*. The calculated molecular weight and pI for BgcGC was estimated to be 86.8 kDa and 5.18, respectively. BlastP program in the NCBI server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) shows the highest identity of BgcGC protein sequence towards GH family 53 protein from *Geobacillus* sp. (75 – 100%), followed by *Bacillus* sp. (63 – 67%). BgcGC belongs to GH family 53 grouped under Clan GH-A, possess a $(\beta/\alpha)_8$ structure as the catalytic domain. Glu361 and Glu479 as indicated in Figure 1 are the two catalytic residues of BgcGC that are conserved in other galactanases [23].

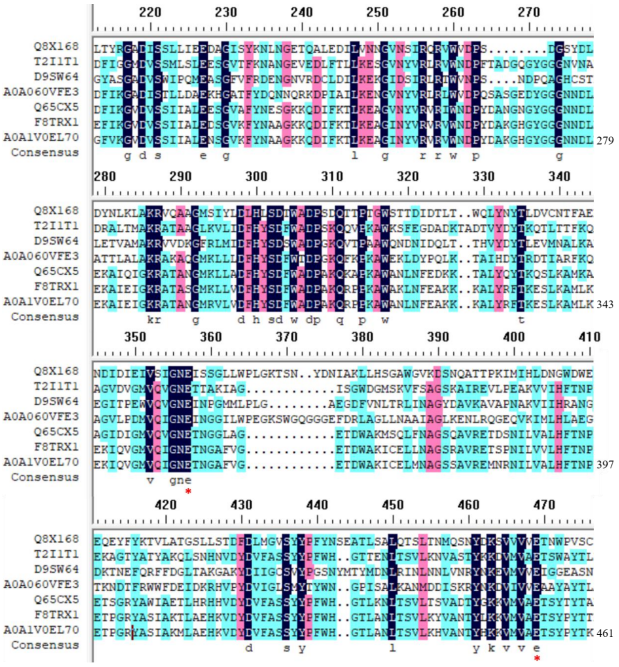


Figure 1 Protein alignment of the core region of catalytic domain between BgcGC and other bacterial and fungal galactanases. UniProtKB accession number represents the origin of the enzyme; Q8X168 (*Aspergillus niger*), T2I1T1 (*Bifidobacterium longum*), D9SW64 (*Clostridium cellulovorans*), A0A060VFE3 (*Klebsiella pneumoniae*), Q65CX5 (*Bacillus licheniformis*), F8TRX1 (*G. stearothermophilus*) and A0A1V0EL70 (*G. mahadii*). Conserved residues are shown on black background, while residues with high similarity are indicated on turquoise and magenta background. The catalytic residues of galactanase are marked below the sequence with a red star (E: Glutamate)

Bacterial galactanase was found to have longer loop region between the βa motifs 7 and 8 compared to fungal galactanases [24]. Due to this, bacterial galactanases prone to hydrolyze longer oligosaccharides bind at -3 and -4 subsites. BgcGC was suggested to have this feature in accordance to beta-1,4-galactanase from *G. stearothermophilus* which shares 99.5% sequence identity [25]. However, recent study revealed a beta-1,4-galactanase from *Bacteroides thetaiotaomicron* resembled the fungal

galactanase mode of action by acting on shorter GOS with degree of polymerization of three and less [9].

3.2 Purification and biochemical characterization of BgcGC

The recovery of purified BgcGC from two-step purification process was evaluated as listed in Table 1. A 20 ml crude extract obtained from a 400 ml culture gave a 20.4% yield of BgcGC with an increase in specific activity up to 11-fold. An approximately 86.8 kDa of purified BgcGC was resolved as a single band on SDS-PAGE (Figure 2). Beta-1,4-galactanase from bacteria sp. has wide range of size from 40 kDa [3] and can be up to 90 kDa [26] compared to the one isolated from fungi (32–49 kDa) [27].

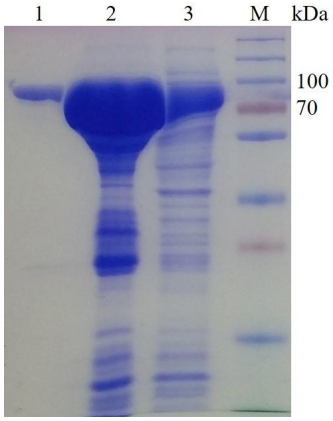


Figure 2 SDS-PAGE of a purified BgcGC (~86.8 kDa). Lane 1: Purified BgcGC after two-steps purification, Lane 2: Concentrated BgcGC after first-step of Ni-NTA purification, Lane 3: Crude extract and Lane M: NEB protein marker

The purified BgcGC displayed optimum activity at 60°C and remained stable from 20–60°C with at least 60% of the activity retained after 30 min pre-incubation at the respective temperature (Figure 3a). BgcGC was derived from a thermophilic gram-positive bacteria in Sungai Klah Hot Springs, Sungkai, Perak, Malaysia at 90°C. Although the bacteria can grow up to 90°C, it is not necessarily be the optimal temperature for the growth [28]. Besides, proteomic analysis of thermophiles exhibited noticeable proteins at significantly lower temperature than the optimal growth temperature [29]. The optimized codon applied for the synthesis of BgcGC might influence the enzyme thermal property. It was suggested the codon usage affect the translational rate of protein which subsequently influence structure arrangement and dynamic motion of the protein [30]. The optimum temperature of beta-1,4-galactanase can be as low as 37°C which originated from *Bifidobacterium longum* [26] and high as 90°C which originated from hyperthermophilic *Thermotoga maritima* [31]. Optimum pH for BgcGC was at 6 and stable at a wide range of pH from 4 to 10 (Figure 3b). According to previous report, optimum pH for most of the bacterial galactanases were in a range of alkaline to neutral [5] compared to fungal galactanases which were more acidic [24, 10]. Variations in the amino acid compositions in the active site proved to have an influence in determining the optimum pH of the enzyme [32].

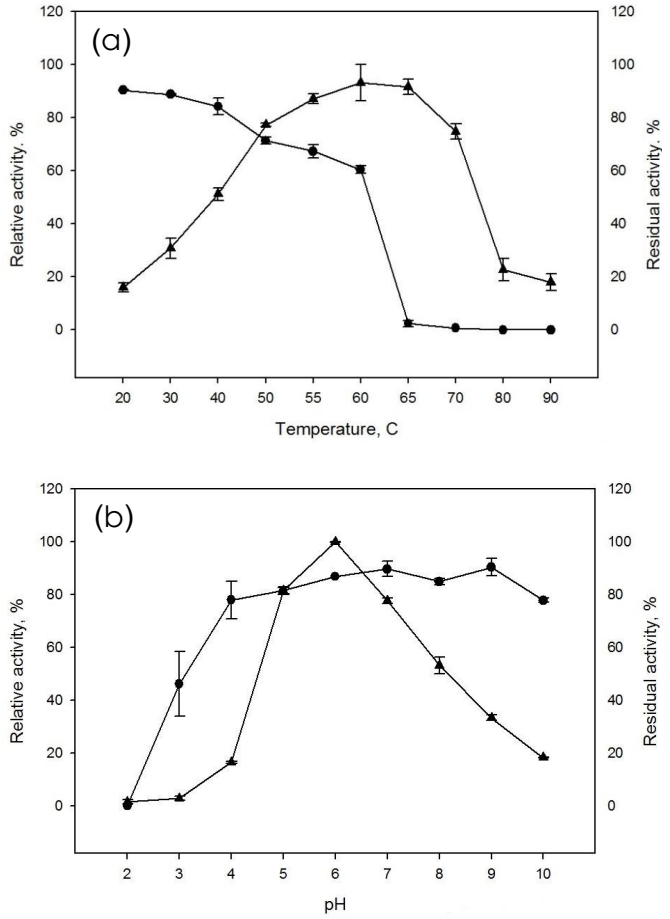


Figure 3 Effect of (a) temperature and, (b) pH on the purified BgcGC activity and stability. Error bars represent the standard deviations of triplicate experiments

Thermal inactivation of BgcGC revealed the half-life of the enzyme became shorter after prolonged exposure to higher temperature (Table 2). BgcGC managed to withstand 2.6 min at 70°C before the enzyme activity drastically reduced. At 60°C, BgcGC half-life was about 35 min compared to beta-1,4-galactanase from *Emericella nidulans,* 10 min [10] and *Talaromyces stipitatus*, two min before increasing to 15 min after the mutation [33]. A delicate interaction of non-covalent forces such as hydrogen bonds, hydrophobic and Van der Waals interactions are holding the protein structure. When exposed to a high temperature, these interactions were disrupted and the protein slowly lose its native conformational form followed by aggregation and precipitation. Protein unfolding caused by heat usually is an irreversible reaction, however in certain cases reversible unfolding can be triggered by the

presence of additive such as glycinamide and methanethiosulphonate [34, 35].

Table 2 Thermal deactivation constants (K_d) and half-lives ($T_{1/2}$) values at various temperatures.

Temperature (°C)	K_d (min^{-1})	$T_{1/2}$ (min)
20	0.0048	144.4
30	0.0063	110.0
40	0.0091	76.2
50	0.0141	49.1
60	0.0196	35.4
70	0.2638	2.6

In this study, BgcGC has been tested with several metal ions at various concentrations to investigate the possible inhibitory or stimulatory effect on the enzyme activity. Figure 4 shows BgcGC activity was severely affected at 5 mM Fe^{2+}. Strong inhibiton caused by Fe^{2+} was also being observed in beta-1,4-galactanase from *Aspergillus sojae* [36] and *Bacillus licheniformis* [5]. BgcGC managed to maintain at least 88% of the activity in various concentrations of K^+. Meanwhile, BgcGC activity demonstrated gradual decrease proportional with the increment of Ca^{2+}, Mg^{2+} and Mn^{2+} concentrations. More than 65% of BgcGC activity was detected in 5 mM Co^{2+}, Cu^{2+}, and Zn^{2+}. However, a sudden drop of activity was observed for these metal ions at 50 and 100 mM concentrations. On the other hand, a slight increment (p>0.05) of BgcGC activity was exhibited at 5 mM Ni^{2+} while higher concentrations abruptly affected the activity. Above all, BgcGC did not show a characteristic of a metalloenzyme or metal-activated enzyme as the activity was not dependent by the presence of metal ions.

According to Figure 5, adverse effect on BgcGC activity was contributed by sodium dodecyl sulphate (SDS) followed by PMSF. In contrast, ethylenediaminetetraacetic acid (EDTA), Tween-20 and 2-mercaptoethanol (2-ME) have no significant effect (p>0.05) on the enzyme activity. Generally, SDS was known as a stronger detergent compared to Tween-20. Thus, dropped in BgcGC activity might be due to SDS molecules interfering with protein conformation and caused the enzyme to lose its active form [37].

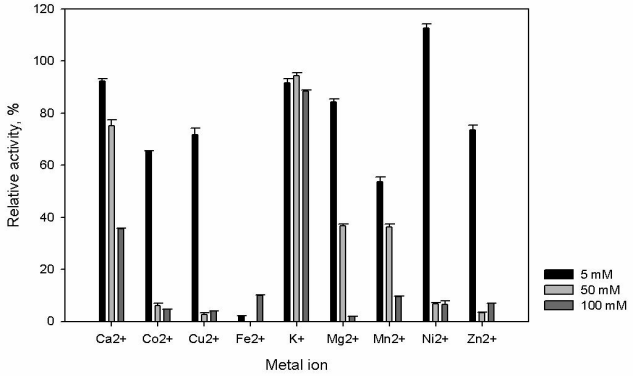


Figure 4 Effect of metal ions on BgcGC activity. Error bars represent the standard deviations of triplicate experiments

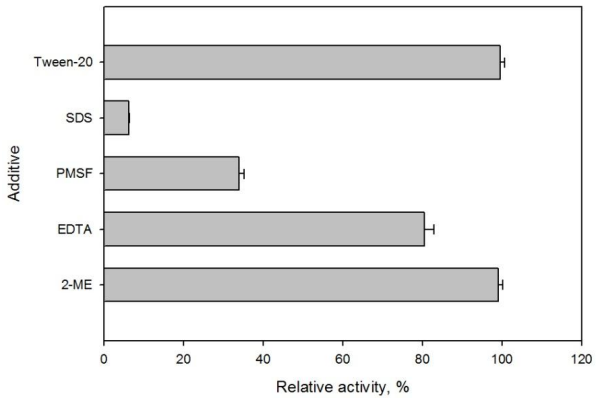


Figure 5 Effect of additives on BgcGC activity. Error bars represent the standard deviations of triplicate experiments

Michaelis-Menten enzyme kinetics were applied to evaluate BgcGC dynamics towards different substrate at various concentrations in standard activity assay. Table 3 shows reaction rate of BgcGC was higher for potato galactan as displayed by higher V_{max} (606.4 umol mg^{-1} min^{-1}) although the higher affinity was towards lupin galactan as revealed by lower K_m value (0.3 mg ml^{-1}). V_{max} and K_m are independent as explained by $K_m = (k_{-1}+k_2)/k_1$ and $V_m = k_2*[E]_0$ equations. This means, V_{max} is not directly related to K_m. K_m values of 0.2 mg/ml [25] and 2.3 mg/ml [5] were reported for galactanase against potato galactan. Nevertheless, higher turnover number, or k_{cat}, was observed for potato galactan which implied more galactan was converted into product per second under optimal conditions compared to other substrates.

On the other hand, BgcGC hydrolyzed lupin galactan more efficiently than potato galactan as demonstrated by the higher enzyme efficiency value or k_{cat}/K_m. The difference of the catalytic efficiency between these two galactans is proposed due to their variation in sizes or structures. Galactose monomers that build up galactan are speculated to be in a linear form or consisted of branch. It is suggested that the galactan oligomer, as part of the pectin complex was influenced by the huge diversity of pectin structure and component which varies depending on the source, time of harvest and extraction method being used [38]. According to the manufacturer of the substrate, potato galactan was likely to be about 100 kDa in size compared to 1182 kDa of lupin galactan. It is inferred that the substrate size caused the difference in the catalytic efficiency as happened to two subclasses of hydrolases although both enzymes cleave the C-N bond [39]. They claimed, increase in the molecular weight of substrate resulted in tighter binding and more efficient enzyme. BgcGC showed less binding affinity and catalytic efficiency towards debranched arabinan, rhamnogalacturonan and pectin.

Table 3 BgcGC kinetic parameters for different substrates

Substrate	V_{max} (umol mg^{-1} min^{-1})	K_m (mg ml^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (ml mg^{-1} s^{-1})
Potato galactan	606.4	1.0	877.5	873.8
Lupin galactan	402.4	0.3	582.3	1694.4
Debranched arabinan	188.0	6.5	272.1	41.7
Rhamnogalacturonan I	2.6	1.3	3.7	3.0
Pectin from apples	87.3	5.1	126.4	24.8

3.3 Hydrolysis of SPP pectin by BgcGC

The presence of galactan oligomer in the SPP pectin was suggested to be a part of rhamnogalacturonan I as confirmed by the detection of GalA, Gal, Rha and Ara (Figure 6). These sugars are the major constituent of rhamnogalacturonan I found in pectin extract from various plant sources such as potato pulp, pumpkin and ginseng [4, 40–42].

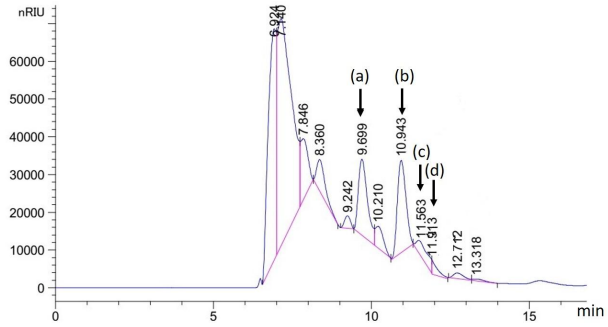
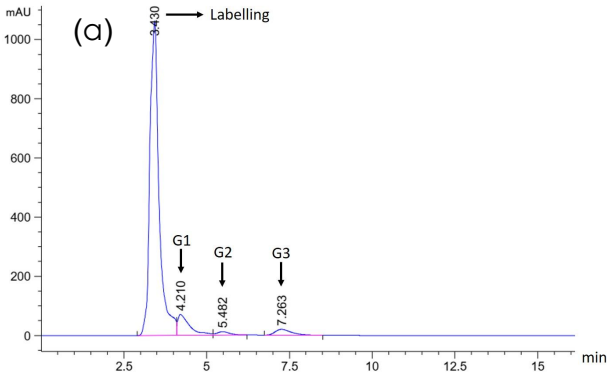


Figure 6 HLPC-RID chromatogram of monosaccharide composition of SPP pectin after performing TFA hydrolysis. Arrowed from left, a) GalA, b) Gal, c) Rha, d) Ara

Reaction of BgcGC towards the treated SPP pectin with endo-PG resulted in the production of galactose and GOS (Figure 7a). Neither galactose nor GOS was detected in SPP pectin treated with endo-PG alone (Figure 7b). This result indicates that the SPP pectin holds the galactose in the form of galactan oligomer. Thus, BgcGC plays an important role as one of the enzyme mixtures in pectin degradation.



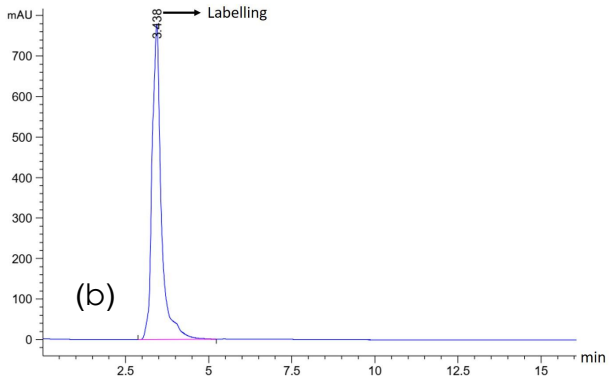


Figure 7 HLPC-UV chromatogram for GOS detection from treated SPP pectin sample with (a) Endo-PG and BgcGC, (b) Endo-PG. G1: Galactose, G2: Galactobiose, G3: Galactotriose, Labelling: ABEE labelling reagent

4.0 CONCLUSION

In summary, BgcGC has been characterized as a thermostable enzyme up to 60°C and possess a wide range of pH stability. This study also conveys the potential of BgcGC to be part of the collective enzymes working together in the degradation of complex pectin polysaccharide and produce specific targeted product of GOS.

Acknowledgement

This research was supported by the Fundamental Research Grant Scheme (Grant No. 4F993) through Universiti Teknologi Malaysia.

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