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RESEARCH PAPER

Enzymatic Synthesis of Propyl-α-glycosides and Their Application as Emulsifying and Antibacterial Agents

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Abstract Alkyl glycosides have been effectively used in many industries because of their biodegradable, emulsification and antibacterial properties. In this study, the alkyl glycoside of propyl glycosides (PG_n) was synthesized using β-cyclodextrin (β-CD) and 1-propanol through the transglycosylation reaction of recombinant cyclodextrin glycosyltransferase (CGTase) from the *Bacillus circulans* A11. The optimal condition for the synthesis of propyl glycosides consisted of an incubation of 1.5% (w/v) β-CD and 500 U/mL of CGTase in a water/propanol content containing 10% (v/v) 1-propanol at pH 6.0, 50°C for 96 h. Upon analysis of the product at the optimal condition by TLC, at least three products which move faster than glucose were observed. These transferred products were formed with molecular weights of 222.1, 384.1 and 546.4 daltons as determined by mass spectrometry analysis; these values were in accordance with propyl glucoside (PG_1), propyl maltoside (PG_2) and propyl maltotrioside (PG_3), respectively. PG_1 and PG_2 were produced and prepared on a large scale and subsequently purified by preparative TLC. The combined ^{1}H- and ^{13}C-NMR analysis confirmed that the structures of PG_1 and PG_2 were propyl-α-D-glucopyranoside and propyl-α-D-maltopyranoside, respectively. Both PG_1 and PG_2 showed emulsification activity and stability in their formation in water and n-hexadecane. Furthermore, the antibacterial activity of both products was determined and it was found that PG_2 had a higher antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* than that of PG_1.

Keywords: antibacterial activity, cyclodextrin glycosyltransferase, intermolecular transglycosylation, non-ionic surfactant, propyl glycosides

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1. Introduction

Alkyl glycosides are non-ionic surfactants which are composed of the hydrophilic sugar head group connected to the alkyl hydrophobic tail *via* a glycosidic bond. They become dominant components in products of the food, detergent, cosmetic and pharmaceutical industries as a result of their biodegradability, emulsification and antibacterial properties [1]. Moreover, alkyl glycosides can be synthesized by both chemical and enzymatic methods. However, the enzymatic syntheses of alkyl glycosides, as shown from many previous studies, are more advantageous than chemical syntheses. The enzymatic method proceeds under non-toxic and mild conditions by a simple one-step reaction process [2]. As reported, the alkyl glycosides could be produced by several enzymes which differed in the kind and length of the sugars and hydrocarbons involved as well as the configuration of the linkages which depended on the specific action of each enzyme [3-6]. The enzymatic synthesis of alkyl glycosides can be achieved through several types of enzymes as extracted from many organisms, such as β-

mannosidase from *Aspergillus niger* [7], β-galactosidase from *Aspergillus oryzae* [8], dextransucrase from *Leuconostoc mesenteroides* [9] and, as indicated in most reports on α- or β-glucosidase, from various other sources [3-5]. However, the group designated cylclodextrin glycosyltransferase (CGTase, E.C. 2.4.1.19) has been studied less through alkyl glycoside synthesis. CGTase has only been used to elongate the carbohydrate group of commercially available dodecyl-β-D-maltoside *via* its coupling reaction using α-CD as a glycosyl donor [6].

CGTases belong to the α-amylase family, which consists of multifunctional enzymes. CGTases catalyze mainly intra- and inter-transglycosylation reactions (cyclization, coupling and disproportionation), but can also exhibit, to a lesser extent, α-amylase-like activity, hydrolyzing starches into short linear saccharides [6,10]. On their first reactions, CGTases convert starch substrates to non-reducing cyclic maltooligosaccharides called cyclodextrins (CDs) through the cyclization reaction. CDs are applied as stabilizers or solubilizers in the food, pharmaceutical and cosmetic industries for their ability to form inclusion complexes with hydrophobic molecules [11]. Besides the cyclization reaction, CGTases also catalyze a disproportionation reaction by transferring linear α-1,4-linked oligosaccharides to another oligosaccharide molecule; they also catalyze a coupling reaction by opening CD rings and transferring them to acceptors [6,10,11]. Naturally, the CGTase acceptors are saccharides or glycosides, which are very abundant in nature [12-14]. This transglycosylation reaction, transferring from donor to acceptor, proceeds *via* the major catalytic machinery of enzymes for the synthesis of new glycosidic bonds [15]. Thus, from this mechanism, CGTase also catalyzes transglycosylation to various compounds other than saccharides, such as piceid [16] and ascorbic acid [17]. Mathew *et al*. [16] have reported the efficient synthesis of piceid glycosides through disproportionation catalyzed by CGTase from *Bacillus macerans* using maltodextrin as a glycosyl donor and piceid as a glycosyl acceptor. The obtained piceid glycosides showed an increase in absorbability in the digestive tract. Aga *et al*. [17] reported on the use of CGTase from *Bacillus stearothermophilus* in the production of ascorbyl glycosides. The result was a greater stability under oxidative conditions through the coupling reaction using α-CD and ascorbic acid as a glycosyl donor and acceptor, respectively. In addition, CGTase can also use water as acceptor, resulting in a hydrolysis reaction.

In this study, the catalytic mechanisms of CGTase were exploited for the synthesis of a series of propyl glycosides. The CGTase enzyme can produce propyl glycosides that have more than one glucose unit attached. There are numerous reports demonstrating that alkyl glycosides can be used as effective protein-solubilizing surfactants and solubilizing agents for cell membranes [18-20]. Therefore, the aim of this study was to use the pBC recombinant CGTase from *Bacillus circulans* A11 to synthesize propyl glycosides using β-CD as the glycosyl donor and 1-propanol as an acceptor. The influence of various parameters on the production of propyl glycosides was investigated. The structure and biological properties of the propyl glycosides were also studied.

2. Materials and Methods

2.1. Materials

Glucose (G1); maltose (G2); maltotriose (G3); maltotetraose (G4); maltopentose (G5); maltohexose (G6); maltoheptaose (G7); methyl-α-D-glucopyranoside (MG_1); sucrose; α-, β- and γ-CDs; soluble potato starch; corn starch; phenolphthalein, n-hexadecane and bovine serum albumin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mannitol salt agar (MSA), yeast extract and tryptone were obtained from Difco (Bacton Dickinson and company, Sparks, MD, USA). Aquacide II, methanol, ethanol, silica gel 60 F_{254} glass plate (20 cm in height) were purchased from Merck (Darmstadt, Germany). 1-Propanol was obtained from CARLO ERBA Reagents (Val de Reuil, France). The commercial glucose oxidase kit was obtained from Human *mti*-diagnostics GmbH (Idstein, Germany). Other chemicals used were of an analytical grade.

2.2. Bacterial cultivation and enzyme production

Escherichia coli BL21 (DE3) cells harboring the pBC recombinant plasmid, a pET19b-based plasmid containing the CGTase gene with a signal peptide sequence from *Bacillus circulans* A11 (GenBank accession no. AF 302787), were prepared as previously described [21]. The recombinant cells were grown in Luria Bertani (LB) medium containing 100 μg/mL ampicillin at 37°C for 24 h. The expression of recombinant enzymes was induced with 0.2 mM IPTG when the OD_{600} of the culture reached 0.6. After 24 h, the cells were removed and the culture broth containing crude CGTase was collected by centrifugation at 12,000 × *g* at 4°C for 2 h for further purification.

2.3. Purification of CGTase

Recombinant CGTase was purified from the culture broth by starch adsorption [22] and DEAE-Toyopearl 650M column chromatography. Corn starch was dried in an oven at 60°C for 3 h, cooled to room temperature and then gradually added to a culture broth of crude CGTase at 4°C to produce a final concentration of 5% (w/v). After 3 h of continuous stirring, the starch cake was collected by

centrifugation at 8,000 × *g* for 30 min at 4°C and washed twice with TB1 buffer (10 mM Tris-HCl, pH 8.5 containing 10 mM $CaCl_2$). The adsorbed CGTase was eluted from the starch cake by stirring for 30 min with 0.2 M maltose in TB1 buffer (3 × 50 mL per liter of starting broth). The eluted CGTase was collected by centrifugation at 10,000 × *g* for 30 min at 4°C. The CGTase solution was poured into dialysis tubing and concentrated by coating the tubing with a water-absorbing agent (carboxymethylcellulose, Aquacide II), followed by dialyzation against TB1 buffer at 4°C three times before being subjected to DEAE-Toyopearl 650M column chromatography. The column (1.5 × 10 cm) was equilibrated with TB2 buffer (10 mM Tris-HCl, pH 8.0). CGTase was then applied to the column, and unbound proteins were eluted with TB2 buffer. After the column was washed thoroughly with TB2 buffer, bound proteins were eluted from the column using a linear salt gradient of 0 ~ 0.2 M sodium chloride in TB2 buffer. Fractions of 2.0 mL were collected continuously. The protein and CGTase-dextrinizing activity profiles of the eluted fractions were monitored by measuring the absorbance at 280 and 600 nm, respectively. Fractions containing CGTase-dextrinizing activity were pooled for further characterization. The purity of the enzyme from each step in the purification was determined using 7.5% native-PAGE and a Coomassie® blue staining method [23].

2.4. Assay of CGTase activity

2.4.1. Dextrinizing activity

The dextrinizing activity of CGTase was determined by measuring the decrease in absorbance of a starch-iodine complex at 600 nm [24]. The enzyme sample (50 μL) was incubated with 0.15 mL of 0.2% (w/v) soluble potato starch in 0.2 M phosphate buffer, pH 6.0, at 40°C for 10 min. The reaction was stopped by the addition of 2 mL of 0.2 M HCl and 0.25 mL of iodine reagent (0.02% (w/v) I_2) in 0.2% (w/v) KI). The mixture was adjusted to a final volume of 5 mL with distilled water, and the absorbance at 600 nm was measured. For the control, HCl was added before the enzyme sample was taken. One unit of enzyme was defined as the amount of enzyme that produced a 10% reduction in the intensity of the blue color of the starch-iodine complex per min under the described conditions.

2.4.2. Coupling activity

2.4.2.1. Glucose oxidase assay

A glucose oxidase kit was used to measure the enzymatic activity by exchanging β-CD for glucose [25]. The reaction mixture (100 μL) contained 40 μL of 1% (w/v) β-CD, 40 μL of 1% (w/v) cellobiose, 5 μL of the enzyme and 15 μL of 0.2 M phosphate buffer, pH 6.0. The reaction mixture was incubated at 50°C for 5 min and was terminated by boiling. Subsequently, the mixture was incubated with 10 μL of glucoamylase (stock solution, 30 units) in 0.2 M phosphate buffer, pH 6.0, at 40°C for 30 min, and the reaction was stopped by boiling for 5 min. An aliquot (10 μL) from the reaction mixture was then added to 1 mL of glucose oxidase reagent and incubated at 37°C for 5 min before the absorbance was measured at 500 nm. The glucose concentration was calculated from equation (1):

$$\text{Glucose concentration (μmol/mL)} = 5.55 \times (A_{Sample}/A_{Std.}) \quad (1)$$

The absorbance of the standard solution ($A_{Std.}$) was measured by adding 10 μL of a standard glucose solution (5.55 μmol/mL) to 1 mL of glucose oxidase reagent. One unit of glucose oxidase activity was defined as the amount of enzyme that produces 1 μmol of glucose per min under the assay conditions used.

2.4.2.2. Phenolphthalein assay

The phenolphthalein assay measures the disappearance of β-CD in the reaction mixture [26]. A 250 μL portion of the β-CD standard or sample solution was incubated with 750 μL of a phenolphthalein solution for 15 min. The decrease in absorbance at 550 nm caused by complex formation between the dye and β-CD was measured. Conversion of ΔA_{550} to μmoles of β-CD was performed using the β-CD phenolphthalein calibration curve. The disappearance of β-CD in the reaction mixture was calculated from the difference between β-CD concentration at 0 and 24 h incubations with CGTase and acceptors. One unit of activity was defined as the amount of enzyme capable of coupling 1 μmole of β-CD to alcohol per min.

2.5. Protein determination

The protein concentration was determined by Bradford's method [27], using bovine serum albumin (BSA) as a standard.

2.6. Synthesis of alkyl glycosides

2.6.1. Donor specificity

Selection of the appropriate donor was performed by incubating 0.64% (w/v) cellobiose and 75 U/mL CGTase in 20 mM phosphate buffer at pH 6.0 with 0.64% (w/v) of the different glycosyl donors (α-CD, β-CD and γ-CD) in a reaction volume of 250 μL at 50°C for 24 h. The occurred glucose from the CGTase transglycosylation reaction was determined using the glucose oxidase assay and the transfer products were analyzed by thin-layer chromatography (TLC) with the mobile phase of System I as described in

Section 2.7.

2.6.2. Determination of transglycosylation efficiency

The efficiency of the CGTase transglycosylation reaction was determined from the transglycosylation product yield, which was determined from the phenolphthalein assay and by TLC analysis. The transglycosylation reaction with various alcohol types and contents were performed using β-CD as a glycosyl donor. The reaction mixture consisted of 0.64% (w/v) β-CD and various alcohol types and concentrations (methanol, ethanol or 1-propanol: 5 ~ 50% (v/v)) and CGTase (75 U/mL by dextrinizing activity) in 20 mM phosphate buffer at pH 6.0 in a total volume of 250 μL. The reaction mixture was incubated at 50°C for 24 h. The reaction was then terminated and assayed using the phenolphthalein method to determine the stability of CGTase in alcohols. In addition, aliquots from each reaction mixture were withdrawn and analyzed by TLC to determine the transglycosylation yield using the mobile phase of System II as described in Section 2.7.

2.7. TLC analysis

The transfer products were analyzed using Silica gel 60 F_{254} (Merck, Germany) base TLC using a 5:5:3 (v/v/v) ratio of n-butanol/ethyl alcohol/water as a mobile phase solvent (System I) to determine the best donor for the synthesis of transglycosylation products. For the analysis of alkyl glycosides products, mobile phase System II (ethyl acetate/acetic acid/water with a 3:1:1 (v/v/v) ratio) was used. Spots on the TLC plate were visualized by dipping into sulfuric acid/methanol (1:9 (v/v) ratio), followed by drying and heating at 110°C for 20 min. The intensity of the synthesized product spots was quantified using a scanning densitometer and a Quantity One® 1-D analysis program. Glucose and methyl-α-D-glucopyranoside (MG_1) were used as standards.

2.8. Optimization of propyl glycoside production

The optimal conditions for the production of propyl glycosides were decided based on the highest percentage yield of propyl glycoside products as determined from the TLC results. The effects of varying the 1-propanol acceptor (5 ~ 40% (v/v)), β-CD donor concentration (0.3 ~ 1.8% (w/v)), enzymatic units (50 ~ 500 U/mL), incubation time (24 ~ 168 h), temperature (30 ~ 70°C) and pH (4.0 ~ 9.0) were investigated using a sequential approach.

2.9. Large-scale production

To obtain a higher yield of propyl glycoside products for structural analysis and to determine their biological properties, large-scale production of propyl glycosides was conducted in a total volume of 250 mL in a 1,000 mL Erlenmeyer flask. The reaction mixture contained the β-CD donor, 1-propanol acceptor and enzyme, all of which were incubated under the optimum condition according to Section 2.8. After the incubation, the reaction mixture was stopped by boiling and then concentrated using a centrifugal evaporator at 45°C prior to purification of the propyl glycosides by preparative thin-layer chromatography (PLC).

2.10. Purification of propyl glycosides

The concentrated reaction mixture was placed on a PLC plate and developed at ambient temperature using solvent System II. One lane of the reaction mixture on the plate was then cut out and used to detect the propyl glycoside products using a sulfuric acid/methanol (1:9 by vol) developing solution. The preparative plate was dried and heated at 110°C for 20 min. Each product from the PLC plate that had approximately the same relative mobility (R_f) values was scraped separately. The propyl glycoside products were extracted from the adsorbent (silica) by dissolving with methanol for 30 min at room temperature and then centrifuging at 8,000 × *g* at 25°C for 1 h. The methanol was further removed using a rotary evaporator at 45°C until the samples were thoroughly dry.

2.11. Structural analysis of propyl glycosides

2.11.1. Digestion with amylolytic enzyme

The structures of CGTase-catalyzed propyl glycosides were preliminarily characterized by incubating with amylolytic enzymes. Each dried propyl glycoside product was dissolved in 0.2 M acetate buffer, pH 5.5, followed by incubating with α-glucoamylase and α-glucosidase (20 U/mL) at 37°C for 24 h. After incubation, the reaction was stopped by boiling and a 10 μL aliquot from each reaction was then analyzed by TLC (System II).

2.11.2. Mass spectrometry (MS) analysis

The transfer products of interest were dissolved in 50% (v/v) methanol, and their masses were determined using a microTOF spectrometer (Bruker, Germany). The products were introduced into a mass spectrometer for processing in the ESI-TOF-MS system, which ionizes by electrospray ionization (ESI) in the sodium positive ion mode using a capillary voltage of 5,000 volts. A 4.0 L/min flow of nitrogen gas at a temperature of 150°C was used to nebulize the analytic solution to droplets using a nebulizer pressure of 1.0 bar. The ions were detected using linear time-of-flight mass spectrometry (TOF-MS), and the mass to charge ratio (m/z) spectra of the products was detected as pseudo-molecular ion peaks $[M+Na]^+$ from which the molecular weights were calculated using a Bruker Daltonics DataAnalysis 3.4 software program.

2.11.3. Nuclear magnetic resonance (NMR) analysis

Structural identification of the propyl glycosides that were synthesized by CGTase was performed by ^{1}H- and ^{13}C-NMR using a Bruker AVANCE III HD 600 NMR Spectrometer in the Department of Biology, Graduated School of Science, Osaka City University, Japan. The spectrometer was operated at 600 MHz at ambient temperature. Chemical shifts were measured using sodium-4,4-dimethyl-4-sila-pentane sulfonate (DSS) as the internal standard. The purified products (2 mg) were freeze-dried and dissolved in 1 mL of CD_3OD containing 0.1% DSS prior to ^{1}H- and ^{13}C-NMR analysis.

2.12. Characterization of the propyl glycoside products

2.12.1. Emulsification activity and stability

The emulsification activity and stability of the propyl glycoside products were determined according to the method of Cirigliano and Carman [28]. Purified propyl glycosides were dissolved in 4 mL of 0.5 mM Tris-HCl buffer containing 0.05 M $MgSO_4$ at pH 8.0 to a final concentration of 0.5 mg/mL. This solution was added to 100 μL of n-hexadecane in a test tube and mixed continuously on a vortex mixer for 2 min to form an oil-in-water emulsion. The emulsification activity was measured after the mixture was left to stand for 10 min. The activity was determined from the turbidity of an oil-in-water emulsion that was measured using a spectrophotometer at 540 nm. In addition, other commercial non-ionic surfactants, such as Triton™ X-100 and methyl-α-D-monoglucoside (MG_1), were also studied. The highest turbidity resulted when the surfactant was set to 100% emulsification activity. For emulsification stability, the turbidity of an oil-in-water emulsion was measured at 540 nm for 60 min. The log of the absorbance at 540 nm was plotted vs time, and the dissociation constant (K_d) value was calculated from the slope of the line. The most stable emulsion formed with the lowest K_d value and was set to 100% emulsification stability.

2.12.2. Antibacterial activity

Cultures of the following microorganisms were used in the study: Gram-positive *Staphylococcus aureus* ATCC 25923 and Gram-negative *Escherichia coli* ATCC 25922. The method of disc diffusion [29], using both the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) [30], was applied.

2.12.2.1. Disc diffusion technique

Filter paper discs (6 mm in diameter) were impregnated with a solution containing 5 mg of each propyl glycoside in sterile water. The inoculum density for a susceptibility test was adjusted to be equivalent to a 0.5 McFarland standard (1×10^8 CFU/mL). An air-dried disc was placed on the surface of *S. aureus*-inoculated mannitol salt agar and *E. coli*-inoculated LB agar. Standard antibiotics, *i.e.*, 10 μg/disc of ampicillin, 10 μg/disc of penicillin, 30 μg/disc of tetracycline and 1 μg/disc of oxacillin, were also tested as well as sterile water as a negative control. The plates were incubated at 37°C for 24 h. The clear zone of inhibition was calculated by measuring the diameter of the inhibition zone. The readings were taken in three different fixed directions, and an average value of inhibition zone was tabulated [31,32].

2.12.2.2. Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) value was determined in liquid LB medium using the microdilution method in a 96-well microtiter plate by incubating the bacteria in LB broth with variable amounts of the sample being tested. Briefly, the 6-mg starter content of propyl glycosides in 50 μL of sterile water was diluted by a two-fold serial dilution in multiple steps in the microtiter plate, while the 0.5 McFarland culture was also diluted with LB broth in a 1:200 (v/v) ratio. The 50 μL of culture was added to the 96-well microtiter plate. Following 18 h of incubation, 10 μL of resazurin solution was added to each well and the plate was incubated for 2 h. The minimum inhibitory concentration (MIC) value was defined as the lowest concentration of propyl glycoside that prevented a color change of resazurin from blue to pink. Resazurin, a blue dye, becomes pink when it is reduced to resorufin by an oxidoreductase within a medium of viable bacteria. The MIC value was determined in triplicate experiments and was expressed as mg/mL [31,32].

2.12.2.3. Minimal bactericidal concentration (MBC)

The MBC was determined using a series of steps that were undertaken after the MIC test had been completed. The MBC test was performed by sub-culturing each well in which no visible growth occurred (blue color) from a previous MIC test to an agar medium. After 24 h of incubation at 37°C, bacterial growth on the agar plate was evaluated. The lowest concentration at which the propyl glycosides killed whole bacteria was determined to be the MBC value.

3. Results and Discussion

3.1. Donor specificity

The donor specificity of the CGTase was evaluated using α-CD, β-CD and γ-CD as the glycosyl donors in the transglycosylation reaction, with cellobiose as an acceptor. The appropriate glycosyl donor for the CGTase was determined by the glucose oxidase method and TLC

analysis (data not shown). The results indicated that CGTase could transfer α-1,4-linked glucosyl residues from all of the CDs donors to a cellobiose acceptor, which was a β-1,4-glucosidic residue. The highest percentage of the coupling activity was observed when β-CD was used as a donor and resulted in the largest transglycosylation yield at 588.71 μg/mL, as determined by TLC analysis. Judging from the enzyme activity and product yields, β-CD is a suitable glycosyl donor for transglycosylation to an alcohol acceptor. In addition to using β-CD as a glycosyl donor for CGTase, other researchers have reported the use of starch as a glycosyl donor for *Bacillus macerans* CGTase in the synthesis of β-arbutin-α-glucoside and β-arbutin-α-maltoside through its transglycosylation reaction [33]. Aga *et al*. [17] found that *Bacillus stearothermophilus* CGTase could catalyze the transglycosylation reaction using α-CD as a donor for the synthesis of 2-*O*-α-D-glucopyranosyl L-ascorbic acid. Thus, the donor specificity of CGTase depends on the unique property of each CGTase that is used in the catalytic reaction to form a new covalent bond with the acceptor of interest.

3.2. Effect of alcohol length and concentration on the CGTase transglycosylation reaction

To investigate the capability of CGTase in the transglycosylation of alkyl alcohols for the synthesis of alkyl glycosides, the influence of the alcohol length and concentration were studied in a buffer and in a mixture of buffer/alcohol by varying the alcohol length and concentration at the optimal temperature (50°C) and pH (6.0) of this enzyme for 24 h. The alcohols used were 5 ~ 50% (v/v) methanol, ethanol and 1-propanol. Following incubation, the reaction was stopped by boiling and then subjected to the phenolphthalein assay and TLC analysis. In this study, the coupling activity of CGTase in buffer was 0.342 μmol/min/mg, which was set at 100% relative activity. As shown in Fig. 1, when the alkyl chain length and concentration of alcohol increased, the activity of the enzyme decreased dramatically. Many reports have suggested that long alkyl chain lengths and high concentrations of alcohol have a direct effect on the loss of the enzyme activity [3-5,7,9,34]. This could be a consequence of the negative influence of the reduction of water activity, enzyme transition-state destabilization and enzyme conformational modifications, which resulted in a decrease of enzyme activity, including the product yield in the alcoholic solutions. Thus, the alcohol chain length and concentration had an important effect on the transglycosylation activity of CGTase.

The effect of the alcohol chain length and concentration on alkyl glycoside synthesis was also determined by subjecting the sample from the above reaction to TLC analysis (System II). The concentration of alkyl glycosides

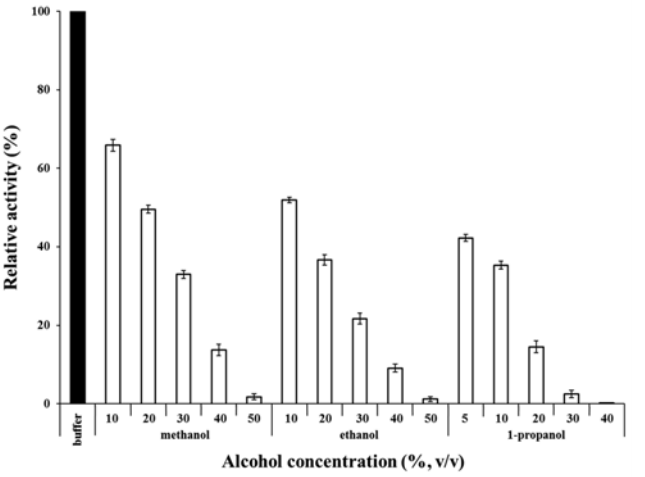

Fig. 1. Relative coupling activity of CGTase in alcohol reaction mixtures. Residual activity (%) was relative to the coupling activity of CGTase incubated with 0.64% (w/w) β-CD and 5 ~ 50% (v/v) alcohols in 20 mM phosphate buffer at pH 6.0, 50°C for 24 h. All values were averaged from two experiments.

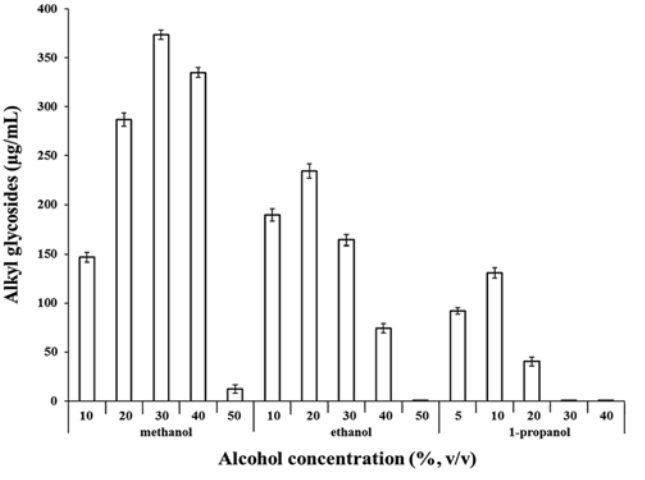

Fig. 2. Alkyl glycoside products from the transglycosylation reaction of β-CD to various alcohol acceptors. Each of alkyl glycoside spots was quantified relative to a standard MG_1 spot in the same TLC plate. All values were averages from duplicate TLC plates.

was calculated from the intensity of the product spots relative to standard MG_1 spots on the same TLC plate and was expressed in units of μg/mL. As shown in Fig. 2, the relationship between the alcohol concentration and yield of product was found to be a bell-shaped curve. This pattern explained the observation that when the concentration of the alcohol as an acceptor substrate in the reaction mixture was increased, the substrate was converted into more products due to the higher turnover rate of the enzyme. However, higher alcohol concentrations decreased the product yield, which may have resulted from the inactivation of the

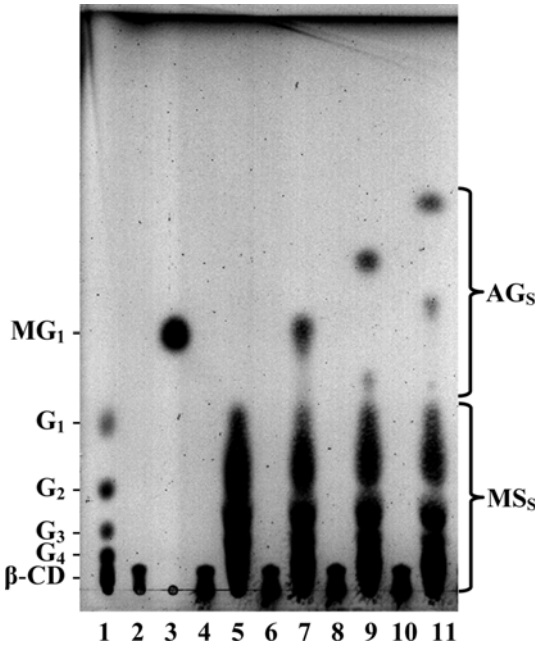

Fig. 3. TLC analysis of the transglycosylation products from β-CD to various alcohols by CGTase. TLC condition was System II, ethyl acetate/acetic acid/water, 3:1:1 (v/v). Lane 1, maltooligosaccharide standards; lane 2, β-CD standard; lane 3, standard MG_1; lane 4-5, product from enzyme reaction without alcohol acceptor at 0 and 24 h; lanes 6-7, product from enzyme reaction between β-CD and 30% methanol at 0 and 24 h; lanes 8-9, product from enzyme reaction between β-CD and 20% ethanol at 0 and 24 h; lanes 10-11, product from enzyme reaction between β-CD and 10% 1-propanol at 0 and 24 h; AGs = alkyl glucosides or alkyl glycosides; MSs = maltooligosaccharides.

CGTase. Therefore, these results indicate that the maximum yield was obtained using 30% (v/v) methanol, 20% (v/v) ethanol or 10% (v/v) 1-propanol.

To ensure that the product observed as a spot on the TLC was an alkyl glycoside product that was produced by CGTase (Fig. 3), a reaction mixture without an alcohol acceptor (Fig. 3, Lane 5) and a reaction mixture containing both substrates incubated with CGTase for 0 h were used as control experiments. After incubating CGTase with the β-CD donor and alkyl alcohol acceptors for 24 h, the expected alkyl glycoside (AG) spots appeared at a higher position than the maltooligosaccharides (MSs) because of the non-polar property of the alkyl chains. In addition to the expected alkyl glycosides, each alcohol acceptor also yielded other hydrolysis products, such as glucose, maltose and other maltooligosaccharides. No alkyl glycoside products were observed in the control experiments. The alkyl glycoside products obtained using 30% (v/v) methanol consisted of at least one product, while with 20% (v/v) ethanol, at least two products were obtained. When 10% (v/v) 1-propanol was used as the acceptor, at least three alkyl glycosides products were detected. Thus, these results demonstrate that the pBC recombinant CGTase could transfer glycosyl residues from β-CD to alkyl alcohols, yielding alkyl glycosides. Furthermore, it is possible that other longer carbohydrate chain glycosides were also produced in reaction mixtures with each alcohol acceptor, but they may have been obscured by the oligosaccharides. Likewise, hydrolysis products were formed with similar R_f values (Fig. 3, Lane 7, 9 and 11). The hydrolysis products resulted from the transfer of β-CD to water, which was due to the multifunctional CGTase. In addition, hydrolysis products occurred in the reaction mixture in preference to alkyl glycosides because of the high affinity of water for the enzyme. In summary, the alkyl glycoside products were produced by catalysis by the recombinant CGTase. This process yielded at least 1-3 products (Fig. 3), which is more than those produced by β-glucosidase. β-glucosidase produced only one alkyl glycoside product from each alcohol substrate [4,5]. To date, the production of propyl glycosides with long chain oligosaccharides by CGTase has never been studied. Thus, 1-propanol was chosen in the present study as a suitable acceptor for further analysis.

3.3. Optimal condition for the synthesis of propyl glycosides

To produce the highest yield of propyl glycosides, the parameters involved in the reaction were optimized in a sequentially independent manner by changing various conditions in the transglycosylation reaction. These parameters included the substrate concentration, enzyme concentration, incubation time, temperature and pH. The products that were obtained from each reaction were analyzed by TLC (System II) and quantified by comparison with the spot intensity of the standard MG_1. As a result, the optimum conditions that were obtained for the synthesis of propyl glycosides were: incubation with 10% (v/v) 1-propanol and 1.5% (w/v) β-CD with 500 U/mL CGTase in 20 mM phosphate buffer, pH 6.0, at 50°C for 96 h (Fig. 4). Under these conditions, the propyl glycoside products comprised up to 37% (w/w) of the total products, which was an increase of greater than 3.5-fold compared to the yield before optimization. This increase in product yield was an increase in all propyl glycosides, including both propyl glucoside and propyl maltooligoside products. The synthesis of propyl glucosides by the action of other enzymes has, in general, been reported. For example, β-glucosidase from Thai rosewood [4] and almond meal [5] could catalyze the synthesis of propyl β-glucosides with yields of 59 and 93% (w/w), respectively. The reaction of *Leuconostoc mesenteroides* dextransucrase with sucrose and propanol resulted in a 38% (w/w) yield of propyl α-glucoside [9]. However, all propyl glycoside productions have been previously reported as consisting of one unit of

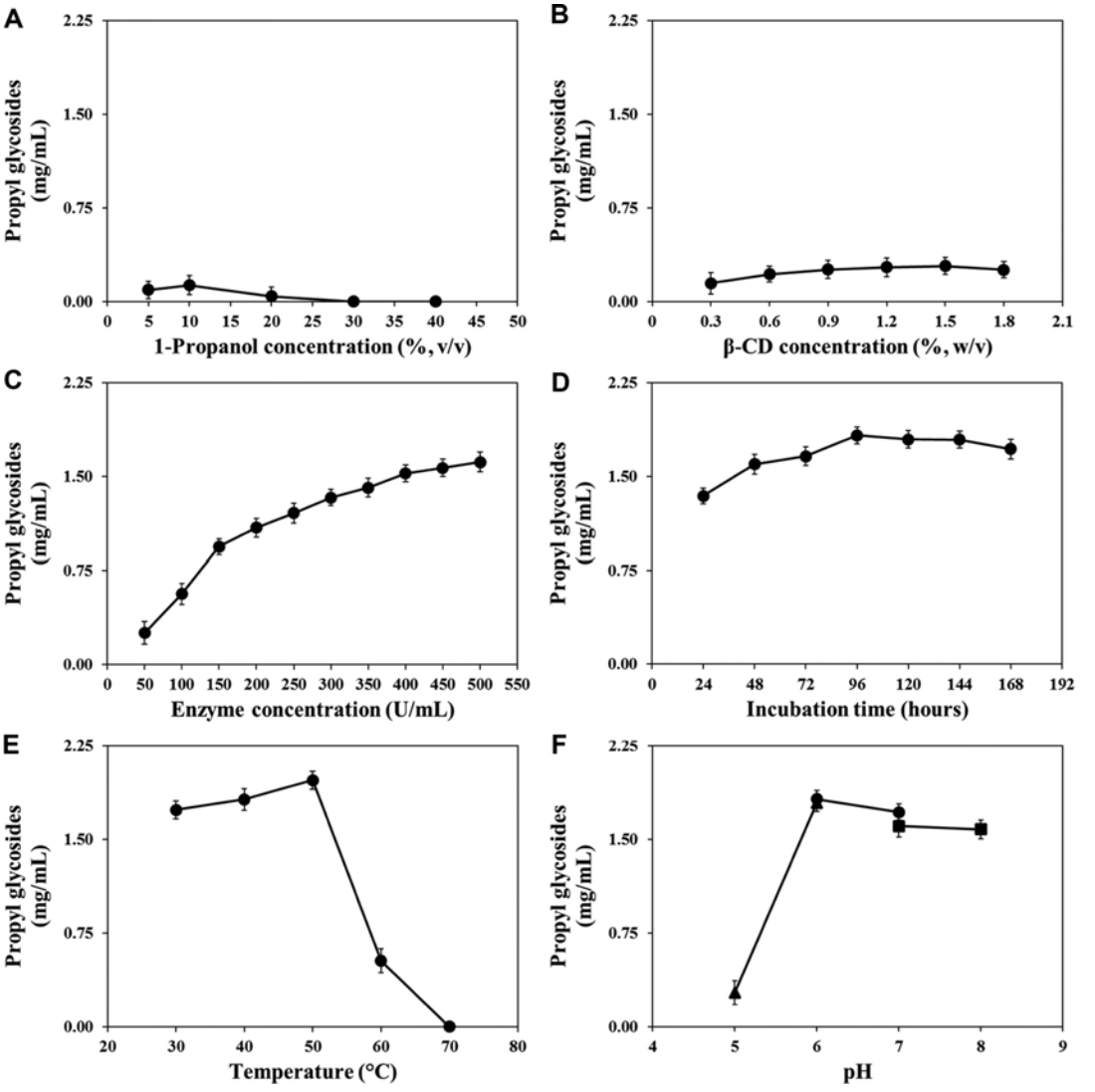

Fig. 4. Effects of (A) 1-propanol concentration, (B) β-CD concentration, (C) enzyme concentration, (D) incubation time, (E) temperature, and (F) pH: acetate buffer (triangle), phosphate buffer (circle), and Tris-HCl buffer (square) on the production of propyl glycosides by CGTase. The amount of propyl glycosides was calculated from the intensity of product spots as compared to 25 μg of standard MG_1 spot.

glucose, which is linked by an α- or β-glycosidic bond. Hence, the propyl glycoside yield that was obtained in this study is quite good compared to the group involved in the bacterial enzyme-catalyzed synthesis.

3.4. Large-scale production and purification of propyl glycosides

The reaction mixture for the synthesis of propyl glycosides was scaled-up to a volume of 250 mL, and the reaction proceeded under the same optimum conditions. Propyl glycoside products from the large-scale production were then purified using PLC. The two propyl glycoside products with different R_f values were scraped separately from the PLC plate and dissolved with methanol. The purified product was applied again onto the TLC plate to determine its purity. The TLC plate results showed that each propyl glycoside product was isolated cleanly, based on their polarities (Fig. 5, Lanes 3 and 4). In a previous study, the synthesis of propyl glucoside from glucose as a glycosyl donor and 1-propanol as an acceptor using almond β-glucosidase has been reported [5]. The propyl glucoside product was determined by high performance liquid chromatography (HPLC) using an Aminex HPX-87H column (300 × 7.8 mm, 9 mm, Bio-Rad). The product was eluted with 5 mmol/L H_2SO_4 at a flow rate of 0.6 mL/min at 65°C and was detected using a refractive index detector, in which propyl monoglucoside was the main product. In our study, however, both monosaccharides and disaccharides

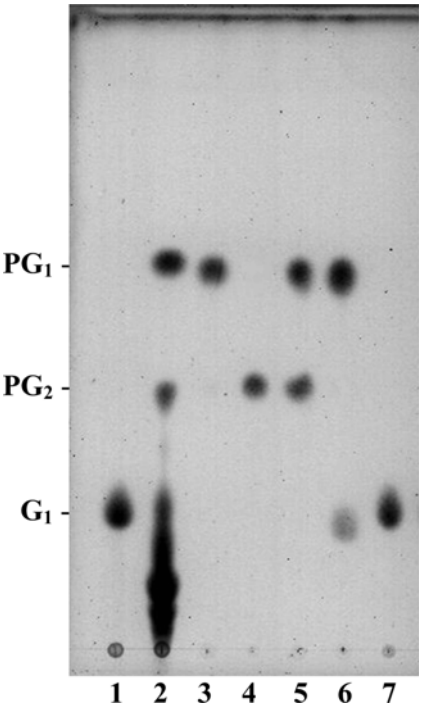

Fig. 5. TLC analysis of the purified propyl glycoside and treatment with enzymes: Lane 1, standard glucose; lane 2, propyl glycosides before purification; lane 3, isolated PG_1; lane 4, isolated PG_2; lane 5, PG_1 and PG_2 mixtures; lane 6, PG_1 and PG_2 mixtures with α-glucoamylase treatment; and lane 7, PG_1 and PG_2 mixtures with α-glucosidase treatment.

of propyl glycoside were observed as major products.

3.5. Mass and structural analysis

3.5.1. Digestion with amylolytic enzymes

The structure of the synthesized propyl glycoside was preliminarily investigated by enzyme treatment of the reaction product with α-glucoamylase (E.C. 3.2.1.3) and α-glucosidase (E.C. 3.2.1.20), followed by TLC analysis of the final deglucosylated products. As shown in Fig. 5, prior to α-glucoamylase digestion, two major glycoside products (PG_1 and PG_2) were observed; however, after digestion with α-glucoamylase, PG_2 was eliminated, while the intensities of PG_1 and the glucose spots increased. This result implied that PG_1 is a propyl monoglycoside, while PG_2 is a propyl diglycoside consisting of disaccharide units with α-1,4-glycosidic linkages. Furthermore, the treatment of propyl glycosides with α-glucosidase produced only one spot of glucose by TLC, while PG_1 and PG_2 were both eliminated. The meaning of these observations is that the first glycosyl residue linked to propyl alcohol is of an α-configuration. Our results are consistent with the fact that CGTase is specific for α-1,4-linkage transfer. Thus, the amylolytic enzymes α-glucoamylase and α-glucosidase could have hydrolyzed the linkage of the transfer product [35].

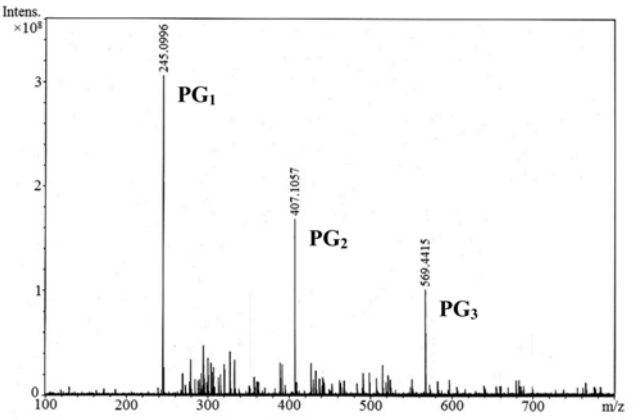

Fig. 6. ESI-TOF mass spectrum of propyl glycosides.

3.5.2. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) analysis

To determine the molecular weights of the products, the obtained transglycosylation products were analyzed using an ESI-TOF mass spectrometer in the positive mode. The pseudomolecular ion peak $[M+Na]^+$ of synthesized propyl glycosides appeared at m/z 245 (222 plus 23 of sodium molecule), m/z 407 (384 plus 23 of sodium molecule) and m/z 569 (546 plus 23 of sodium molecule) (Fig. 6). These results corresponded to the molecular weights of propyl glucoside (PG_1, $C_9H_{18}O_6$), propyl maltoside (PG_2, $C_{15}H_{28}O_{11}$) and propyl maltotrioside (PG_3, $C_{21}H_{38}O_{16}$), respectively.

The structures of the alkyl glycosides were determined by ^{13}C- and ^{1}H-NMR, as shown in Tables 1 and 2. For the PG_1 product, the ^{13}C-NMR spectrum displayed nine carbon signals. Six of these (δ 62.33 - δ 99.97) were assigned to glucose. Another three signals (δ 11.05 - δ 70.70), which increased when combined with glucose while comparing carbon signals of 1-propanol [36], were assigned to the propyl group. The C1' signal of the glucose unit changed significantly from 92.77 to 99.97 ppm (+7.2 ppm) because of the glycosylation of 1-propanol with glucose [37]. The types of glycosidic linkages were also investigated by ^{1}H-NMR and were based on the coupling constant values of the anomeric protons, as shown in Table 2. The PG_1 product gave a double signal at 4.79 ppm with a coupling constant (*J*) of 3.5 Hz, which suggested that the type of glycosidic linkage was an α-configuration. When PG_1 was analyzed in terms of its structure and in combination with its molecular weight, we formed the summary conclusion that the PG_1 product was propyl-α-D-glucopyranoside (Fig. 7A).

Likewise, structural analysis of PG_1 and ^{13}C-NMR spectrum analysis of PG_2 were also performed and revealed

Table 1. ^{13}C-NMR data for PG_1 and PG_2

	Compound			
	1-Propanol[a]	Glucose[b]	PG_1	PG_2
C-Alkyl				
1	59.50		70.70	70.85
2	23.50		23.76	23.13
3	9.98		11.05	10.67
C-Glucose				
1'		92.77	99.97	99.75
2'		72.15	73.38	73.32
3'		73.43	74.86	74.44
4'		70.32	71.65	78.32
5'		72.10	73.57	72.85
6'		61.27	62.33	62.20
1"				97.58
2"				72.47
3"				74.55
4"				71.55
5"				73.51
6"				62.25

Values of chemical shift (δ) are stated in ppm.
[a,b]The chemical shift values are taken from the previous reports [36,37].

Table 2. ^{1}H-NMR chemical shift values and coupling constants for the glucosidic protons

Compound	Glucosidic protons	
PG_1	4.79 (3.5)	
PG_2	4.81 (3.5)	4.84 (3.5)

Values of chemical shift (δ) are stated in ppm. Coupling constants (Hz) are shown in parentheses.

fifteen carbon signals (Table 1). Three of the signals were assigned to the propyl group (δ 10.67 - δ 70.85), while the remaining twelve signals (δ 62.20 - δ 99.75) were derived from two units of glucose. Compared to the sole glucose signals, the C1', C4' and C1" signals in the glucose of PG_2 showed a downfield chemical shift of 6.98, 8.0 and 4.81 ppm, respectively. These downfield shifts suggested that PG_2 had two glycosidic linkages, one of which was between the propyl group and glucose and the other between glucose and glucose. In addition, the ^{1}H-NMR spectrum of PG_2 revealed that a glucosyl or maltosyl residue was transferred to the C1 of 1-propanol to provide an α-anomeric configuration, based on the double signal at 4.81 ppm (J = 3.5 Hz). Similarly, the last glucosyl residue of PG_2 was transferred to the C4' position in the glucose unit of PG_1 with an α-configuration that had been confirmed by a double signal at 4.84 ppm (J = 3.5 Hz) of the anomeric proton by ^{1}H-NMR analysis (Table 2). During the course of integrated NMR and MS analyses, this product was determined to be propyl-α-D-maltopyranoside with an α-1,4 glycosidic linkage

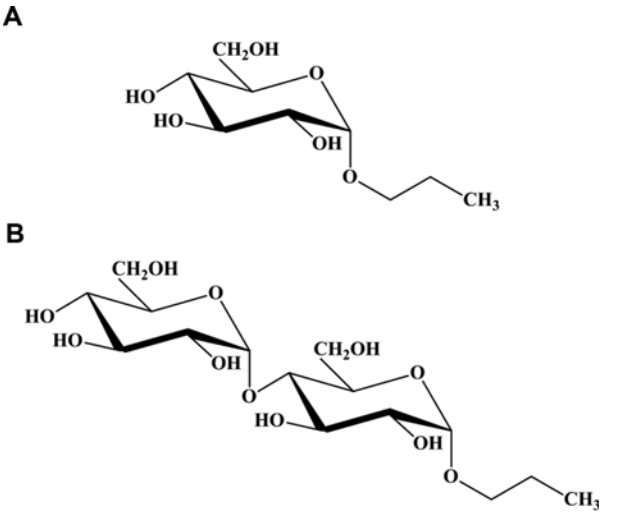

Fig. 7. Structures of (A) propyl-α-D-glucopyranoside and (B) propyl-α-D-maltopyranoside.

(Fig. 7B). To our knowledge, these findings are the first to report the use of ^{1}H and ^{13}C-NMR techniques to resolve the structure of propyl-α-D-glucopyranoside and propyl-α-D-maltopyranoside. Generally, most of the propyl glycosides in previous studies were generated by the action of β-glucosidase from several sources, such as Thai rosewood [4] and almond meal [5]. Other enzymes, such as β-mannosidase from *A. niger* [7], were also used to produce the propyl mannoside. However, the propyl glycosides that were obtained using these enzymes yielded products with β-linkages. Very few studies regarding the synthesis of propyl glycosides with an α-linkage have been reported. For example, *L. mesenteroides* α-dextransucrase was used to produce propyl α-glycoside from sucrose and propanol [9]. However, the product obtained using this enzyme contained only one attached unit of monosaccharide. An in-depth study of this report focused on the syntheses of methyl-α-D-glucopyranoside and ethyl-α-D-glucopyranoside as surfactants. Thus, we were presented with a unique finding in propyl α-glycosides with oligosaccharides that were attached in large amounts. Specifically, we learned that CGTase from recombinant pBC cells is a suitable enzyme that acts differently from other enzymes in the transglycosylation of oligosaccharides to 1-propanol for the synthesis of propyl glycosides.

3.6. Characterization of the propyl glycoside products

3.6.1. Emulsification activity and stability

The emulsification activity of purified propyl glycosides was determined from their ability to form an oil-in-water emulsion with n-hexadecane and was also used to judge their surfactant properties. The highest turbidity was

Table 3. Emulsification activity and stability of the commercial products and transglycosylated products

Compound	Emulsification activity[a] (%)	Emulsification stability[b] (%)
Triton™ X-100	100	100
MG_1	6 ± 1.4	83.7 ± 1.1
PG_1	30 ± 1.6	87.2 ± 0.9
PG_2	36 ± 1.2	91.1 ± 1.1

[a]Determined by the turbidity of emulsification.
[b]Calculated from the logarithmic plot of absorbance versus time.
All values are presented as the mean ± SD from three separate experiments.

observed in the reaction mixture with Triton™ X-100 and was set as 100% emulsification activity. The turbidity of other glycosides was measured and compared to that with Triton™ X-100. As shown in Table 3, the results show that the emulsification activity of PG_1 and PG_2 was 30 and 36%, respectively, compared to that of Triton™ X-100. In addition, these synthesized glycoside products have better emulsifying properties than commercial methyl glycoside (MG_1) and exhibited a higher emulsification activity than MG_1. These results suggest that the emulsification activity of alkyl-α-D-glycosides could be affected by the alkyl and carbohydrate chain lengths. The longer chains of both alkyl and carbohydrate groups exhibited better activity with n-hexadecane. These observations can be summarized by noting that the emulsion-forming properties are related to the physiochemical structure of an emulsifier. In addition to the emulsification activity, the emulsification stability was also determined using the dissociation constant (K_d) value. The results obtained demonstrated that the emulsification stability of Triton™ X-100 was the best, followed by propyl-α-D-diglucoside, propyl-α-D-monoglucoside and methyl-α-D-monoglucoside. These data indicate that propyl-α-D-glycosides can be used as an emulsion-stabilizing agent. It is highly probable, as was reported previously, that the emulsification activity of alkyl glycosides increases according to the length of the hydrophobic alkyl chains and the number of glucose groups. Furthermore, the alkyl chain length has a much stronger influence on emulsification activity compared to the number of glucose groups in the alkyl glycoside [38].

3.6.2. Antibacterial activity

The antibacterial activity of the synthesized propyl glycosides was determined by the disc diffusion method. The results are expressed as the diameter of the inhibition zone (Table 4). The study revealed that both propyl glucoside and propyl maltoside (5 mg/disc) exhibited moderate antibacterial activity against *S. aureus*, with inhibition zones of 10 and 12 mm, and inhibited growth of *E. coli*, with inhibition zones of 12 and 14 mm, respectively. As seen in Table 5, the lowest MIC and MBC values for PG_2 were found to be 1.88 and 3.75 mg/mL against *S. aureus*, respectively, and 1.88 and 1.88 mg/mL against *E. coli*, respectively. Moreover, as a result, it showed that PG_2 had greater antibacterial activity than PG_1 and was the most effective agent for inhibiting *E. coli* growth. This pattern of activity of the propyl glycosides is likely a result of their properties as non-ionic surfactants that can disrupt both the lipopolysaccharides and phospholipids of the gram-negative bacterial cell wall, resulting, in turn, in the inhibition of bacterial growth. Similarly, Matin *et al*. [39] and Matsumura *et al*. [1] found that other alkyl glycosides, such as butyl glycoside and dodecyl mannoside, exhibited a broad spectrum of anti-

Table 4. Antibacterial activity of propyl glycoside products and standard antibiotic drugs

Microorganism	Antibacterial activity (Zone of Inhibition in mm of diameter[a])					
	AMP[b] (10 μg)	TET[b] (30 μg)	PEN[b] (10 μg)	OXA[b] (1 μg)	PG_1 (5 mg)	PG_2 (5 mg)
S. aureus ATCC 25923	30 ± 0.2	26 ± 0.1	40 ± 0.2	24 ± 0.1	10 ± 0.1	12 ± 0.1
E. coli ATCC 25922	22 ± 0.1	24 ± 0.1	0	0	12 ± 0.2	14 ± 0.1

[a]Values of inhibitory zone in mm are the mean ± SD of three parallel measurements.
[b]The concentration of antibiotics (AMP, Ampicillin; TET, Tetracycline; PEN, Penicillin; OXA, Oxacillin) was used according to the standard concentration shown in CLSI document [29].

Table 5. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of propyl glycosides against microorganisms

Microorganism	MIC and MBC values[a]		
	Ampicillin (μg/mL)	PG_1 (mg/mL)	PG_2 (mg/mL)
S. aureus ATCC 25923	0.08 ± 0 (0.08 ± 0[b])	3.75 ± 0 (3.75 ± 0)	1.88 ± 0 (3.75 ± 0)
E. coli ATCC 25922	0.98 ± 0 (0.98 ± 0)	3.75 ± 0 (3.75 ± 0)	1.88 ± 0 (1.88 ± 0)

[a]All data are shown as mean ± SD derived from triplicate experiments.
[b]MBC values are shown in parentheses.

microbial activity. They reported that the antimicrobial activity resulted from the dominant role of alkyl glycosides in penetrating microbial cell membranes [19,20,40]. In addition, PG_2 consisted of two glucose residues that enhanced both the water solubility and surface activity [41,42]. Thus, PG_2 is more effective than PG_1 as an antibacterial agent. In contrast, an antibiotic, ampicillin, had a propensity to inhibit peptidoglycan synthesis in the cell wall of gram-positive bacteria (Table 5).

4. Conclusion

The enzyme from the pBC recombinant cell containing the CGTase gene from *Bacillus circulans* A11 can be used to synthesize propyl α-D-glycosides, which has one-to-several glucose residues from the glycosyl donor β-CD and 1-propanol as acceptor through a transglycosylation reaction. Under optimal conditions, the yield of propyl glycosides obtained was 37% (w/w) of the total products. The acceptor products for 1-propanol were confirmed as propyl-α-D-glucopyranoside and propyl-α-D-maltopyranoside by ESI-TOF-MS and NMR analyses. Moreover, the products obtained from this study also exhibited an emulsification potential and antibacterial activity against *E. coli* and *S. aureus*.

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References

1. Matsumura, S., K. Imai, S. Yoshikawa, K. Kawada, and T. Uchibor (1990) Surface activities, biodegradability and antimicrobial properties of n-alkyl glucosides, mannosides and galactosides. *J. Am. Oil Chem. Soc*. 67: 996-1001.
2. van Rantwijk, F., M. W. V. Oosterom, and R. A. Sheldon (1999) Glycosidase-catalysed synthesis of alkyl glycosides. *J. Mol. Catal. B-Enz*. 6: 511-532.
3. Ito, J., T. Ebe, S. Shibasaki, H. Fukuda, and A. Kondo (2007) Production of alkyl glucoside from cellooligosaccharides using yeast strains displaying *Aspergillus aculeatus* β-glucosidase 1. *J. Mol. Catal. B-Enz*. 49: 92-97.
4. Lirdprapamongkol, K. and J. Svasti (2000) Alkyl glucoside synthesis using Thai rosewood β-glucosidase. *Biotechnol. Lett.* 22: 1889-1894.
5. Wang, Q., H. Yu, N. Zhao, C. Li, Y. Shang, H. Liu, and J. Xu (2012) Significantly improved equilibrium yield of long-chain alkyl glucosides *via* reverse hydrolysis in a water-poor system using cross-linked almond meal as a cheap and robust biocatalyst. *Chin. J. Catal*. 33: 275-280.
6. Svensson, D., S. Ulvenlund, and P. Adlercreutz (2009) Efficient synthesis of a long carbohydrate chain alkyl glycoside catalyzed by cyclodextrin glycosyltransferase (CGTase). *Biotechnol. Bioeng*. 104: 854-861.
7. Itoh, H. and Y. Kamiyama (1995) Synthesis of alkyl β-mannosides from mannobiose by *Aspergillus niger* β-mannosidase. *J. Ferment. Bioeng*. 80: 510-512.
8. Ooi, Y., T. Hashimoto, N. Mitsuo, and T. Satoh (1985) Enzymic formation of β-alkyl glycosides by β-galactosidase from *Aspergillus oryzae* and its application to the synthesis of chemically unstable cardiac glycosides. *Chem. Pharm. Bull*. 33: 1808-1814.
9. Kim, Y. -M., B. -H. Kim, J. -S. Ahn, G. -E. Kim, S. -D. Jin, T. -H. Nguyen, and D. Kim (2009) Enzymatic synthesis of alkyl glucosides using *Leuconostoc mesenteroides* dextransucrase. *Biotechnol. Lett*. 31: 1433-1438.
10. van der Veen, B. A., G.-J. W. M. van Alebeek, J. C. M. Uitdehaag, B. W. Dijkstra, and L. Dijkhuizen (2000) The three transglycosylation reactions catalyzed by cyclodextrin glycosyltransferase from *Bacillus circulans* (strain 251) proceed *via* different kinetic mechanisms. *Eur. J. Biochem.* 267: 658-665.
11. Loftsson, T., P. Jarho, M. Másson, and T. Järvinen (2005) Cyclodextrins in drug delivery. *Expert Opin. Drug Deliv.* 2: 335-351.
12. Kitahata, S., K. Hara, K. Fujita, H. Nakano, N. Kuwahara, and K. Koizumi (1992) Acceptor specificity of cyclodextrin glycosyltransferase from *Bacillus stearothermophilus* and synthesis of α-D-glucosyl *O*-β-D-galactosyl-(1→4)-β-D-glucoside. *Biosci. Biotechnol. Biochem*. 56: 1386-1391.
13. Kometani, T., Y. Terada, T. Nishimura, H. Takii, and S. Okada (1994) Transglycosylation to hesperidin by cyclodextrin glucanotransferase from an alkalophilic *Bacillus* Species in alkaline pH and properties of hesperidin glycosides. *Biosci. Biotechnol. Biochem*. 58: 1990-1994.
14. Wongsangwattana, W., J. Kaulpiboon, K. Ito, and P. Pongsawasdi (2010) Synthesis of cellobiose-containing oligosaccharides by intermolecular transglucosylation of cyclodextrin glycosyltransferase from *Paenibacillus* sp. A11. *Proc. Biochem*. 45: 947-953.
15. Mosi, R., S. He, J. Uitdehaag, B. W. Dijkstra, and S. G. Withers (1997) Trapping and characterization of the reaction intermediate in cyclodextrin glycosyltransferase by use of activated substrates and a mutant enzyme. *Biochem*. 36: 9927-9934.
16. Mathew, S., M. Hedström, and P. Adlercreutz (2012) Enzymatic synthesis of piceid glycosides by cyclodextrin glucanotransferase. *Proc. Biochem*. 47: 528-532.
17. Aga, H., M. Yoneyama, S. Sakai, and I. Yamamoto (1991) Synthesis of 2-*O*-α-D-glucopyranosyl L-ascorbic acid by cyclomaltodextrin glucanotransferase from *Bacillus stearothermophilus*. *Agric. Biol. Chem*. 55: 1751-1756.
18. Aoudia, M. and R. Zana (1998) Aggregation behavior of sugar surfactants in aqueous solutions: effects of temperature and the addition of nonionic polymers. *J. Colloid Interface Sci*. 206: 158-167.
19. Knol, J., K. Sjollema, and B. Poolman (1998) Detergent-mediated

reconstitution of membrane proteins. *Biochem*. 37: 16410-16415.
20. Lichtenberg, D., H. Ahyayauch, and F. M. Goñi (2013) The mechanism of detergent solubilization of lipid bilayers. *Biophys. J*. 105: 289-299.
21. Kaulpiboon, J., W. Prasong, V. Rimphanitchayakit, S. Murakami, K. Aoki, and P. Pongsawasdi (2010) Expression and characterization of a fusion protein-containing cyclodextrin glycosyltransferase from *Paenibacillus* sp. A11. *J. Basic Microbiol*. 50: 427-435.
22. Kato, T. and K. Horikoshi (1984) Immobilized cyclomaltodextrin glucanotransferase of an alkalophilic *Bacillus* sp. No. 38-2. *Biotechnol. Bioeng*. 26: 595-598.
23. Weber, K. and M. Osborn (1975) Proteins and SDS: Molecular weight determination on polyacrylamide gels and related procedures. pp. 179-233. In: H. Neurath, R. L. Hill, and C. Border (eds.). *The Proteins*. Academic Press, NY, USA.
24. Fuwa, H. (1954) A new method for microdetermination of amylase activity by the use of amylose as the substrate. *J. Biochem*. 41: 583-603.
25. Miwa, I., J. Okudo, K. Maeda, and G. Okuda (1972) Mutarotase effect on colorimetric determination of blood glucose with -D-glucose oxidase. *Clin. Chim. Acta* 37: 538-540.
26. Goel, A. and S. N. Nene (1995) Modifications in the phenolphthalein method for spectrophotometric estimation of beta cyclodextrin. *Starch*. 47: 399-400.
27. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 72: 248-254.
28. Cirigliano, M. C. and G. M. Carman (1984) Isolation of a bioemulsifier from *Candida lipolytica*. *Appl. Environ. Microbiol*. 48: 747-750.
29. CLSI (2014) Performance standards for antimicrobial susceptibility testing; Twenty-fourth informational supplement. M100-S24.
30. Sarker, S. D., L. Nahar, and Y. Kumarasamy (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods*. 42: 321-324.
31. Awwad, A. M., N. M. Salem, and A. O. Abdeen (2013) Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. *Int. J. Ind. Chem*. 4: 1-6.
32. Bhalodia, N. R. and V. J. Shukla (2011) Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. *J. Adv. Pharm. Technol. Res*. 2: 104-109.
33. Sugimoto, K., T. Nishimura, K. Nomura, K. Sugimoto, and T. Kuriki (2003) Syntheses of arbutin-α-glycosides and a comparison of their inhibitory effects with those of α-arbutin and arbutin on human tyrosinase. *Chem. Pharm. Bull*. 51: 798-801.
34. Rodríguez, M., A. Gómez, F. Gónzalez, E. Barzana, and A. López-Munguía (1997) Stability of invertase in alcoholysis reactions with methanol. *J. Mol. Catal. B-Enz*. 2: 299-306.
35. Nakamura, A., K. Haga, and K. Yamane (1994) The transglycosylation reaction of cyclodextrin glucanotransferase is operated by a Ping-Pong mechanism. *FEBS Lett*. 337: 66-70.
36. Smith, J. G. (2011) *Organic Chemistry*. 3rd ed., p. 526. McGraw-Hill, NY, USA.
37. Roslund, M. U., P. Tähtinen, M. Niemitz, and R. Sjöholm (2008) Complete assignments of the ^{1}H and ^{13}C chemical shifts and $J_{H,H}$ coupling constants in NMR spectra of D-glucopyranose and all D-glucopyranosyl-D-glucopyranosides. *Carbohyd. Res*. 343: 101-112.
38. von Rybinski, W. (1996) Alkyl glycosides and polyglycosides. *Curr. Opin. Colloid Interface Sci*. 1: 587-597.
39. Matin, M. M., M. M. H. Bhuiyan, and A. K. M. S. Azad (2013) Synthesis and antimicrobial evaluation of some n-butyl α- and β-D-glucopyranoside derivatives. *RGUHS J. Pharm. Sci*. 3: 53-59.
40. Wenk, M. R., T. Alt, A. Seelig, and J. Seelig (1997) Octyl-β-D-glucopyranoside partitioning into lipid bilayers: Thermodynamics of binding and structural changes of the bilayer. *Biophys. J*. 72: 1719-1731.
41. Rather, M. Y. and S. Mishra (2013) An alternative enzyme based method for synthesis of alkyl-glycosides. *Sustain. Chem. Proc*. 1: 1-15.
42. Sułek, M. W., M. Ogorzałek, T. Wasilewski, and E. Klimaszewska (2013) Alkyl polyglucosides as components of water based lubricants. *J. Surfact. Deterg*. 16: 369-375.