Green Technology for One-step Production of Alkyl Glycosides Using Cyclodextrin Glycosyltransferase from *Paenibacillus* sp.RB01

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Abstract. Biological production of alkyl glycosides were performed using enzyme catalytic method instead of the use of chemical catalyst. Cyclodextrin glycosyltransferase (CGTase) from Paenibacillus sp.RB01 was used to catalyze the transglycosylation reaction from a glycosyl donor to an appropriate alcohol acceptor including primary, secondary and tertiary alcohols. The reaction mixtures containing 0.6% (w/v) β-cyclodextrin (β-CD), 2-30 % (v/v) of various alcohols and 200 U/mL of CGTase in 50 mM acetate buffer pH 6.0 were performed at 40 °C for 24 hours with continuous shaking. The effect of alcohols on the transglycosylation activity of CGTase was assayed. It was found that the alcohol length and concentration had significant effect on the enzyme activity. The highest medium chain alkyl glycoside yield was obtained when isobutanol (5% v/v) was used as a glycosyl acceptor. Products were checked to confirm that they were glycoside derivatives by the treatment with glucoamylase and α-glucosidase. The result obtained showed that the product was a glycoside derivative which had an α-linkage between isobutanol and glucose residue. Various carbohydrate sources could be used as glycosyl donor for the process of the alkyl glycoside production.

Keywords: Alkyl glycoside, Cyclodextrin glycosyltransferase, Transglycosylation

1. Introduction

Green Technology has been mentioned in the past few years due to the environmental problems are still increasing as a result of the environmental care products demand increased. The biological processes have thus been developed for any manufacturing steps.

Alkyl glycosides are classified as a non-ionic surfactant which is produced from the reactions of fatty alcohols and saccharides. Therefore, they are rapidly biodegradable and non-toxic. Their structures are amphipathic molecules consisting of one or several units of sugar head group connected via an ether bond with hydrophobic hydrocarbon chain. They have good emulsification activity and wetting properties. Furthermore, the sugar head group could ionize with no charge. Thus, they have potential use as effective agents in membrane solubilization and also in food and cosmetic industries [1]. Enzymatic synthetic method is advantageous over the chemical ones, as they ensure the stereo-selective obtaining of the product. Although it has been shown that their chemical synthesis was well-developed, the method still employs toxic and expensive compounds to yield

α and β-configuration products via several steps. Hence, the biological process of one-step production of alkyl glycosides has been developed using enzyme catalyzed glycosylation reaction [2, 3]. On the other hand, reverse hydrolysis reaction was also employed by controlling water activity in the reactions [4].

Long chain alkyl glycosides have higher surfactant activity than those of the short chain ones. Therefore, they will undoubtedly constitute an important part not only in industrial chemicals widely used in almost every sector of various industries but also in household uses [5]. In 2011, short-chain primary α-alkyl

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glycosides were successfully synthesized through transglycosylation reaction by cyclodextrin glycosyltransferase (CGTase, E.C. 2.4.1.19) from *Paenibacillus* sp. RB01 which was isolated from hot spring area in Thailand [6]. The use of CGTase for alkyl glycoside synthesis is of great interest due to the fact that a glycoside product containing more than one sugar residues could be synthesized. On the other hand, the reaction catalyzed by glycohydrolases was able to transfer only one monosaccharide unit.

In this work, we demonstrated the synthesis of alkyl glycosides from miscible and immiscible alcohols including primary, secondary and tertiary ones through one-step system using CGTase from *Paenibacillus* sp. RB01. Effect of alcohols on the transglycosylation ability of the enzyme was investigated and the product obtained was checked for the anomeric structure by treatment with hydrolytic enzymes. The ability to use various donor substrates was also determined.

2. Materials and Methods

2.1. Enzymes and Chemicals

β-Cyclodextrin, methyl-α-D-glucopyranoside and α-glucosidase were obtained from Sigma-Aldrich Chemical Co., USA. Glucoamylase (amyloglucosidase) was from *Aspergillus niger* and 2-hexanol were purchased from Fluka, Switzerland. Soluble starch (synthesis grade from potato) and flomax® were from Scharlau Chemie S.A., Spain and national starch USA, respectively. Silica gel 60 F_{254} aluminium sheets (0.2 mm), methanol, ethanol, cyclohexanol and 1-octanol were obtained from Merck, Germany. 1-propanol, 2-propanol, and 1-butanol were from Carlo Erba Reagents, Italy. Isobutanol, 2-butanol, 1-pentanol and 1-heptanol were purchased from BDH Chemical, England. 2-pentanol was obtained from Lab Scan, Thailand. Other chemicals were of analytical grade.

2.2. Synthesis of Various Alkyl Glycosides by Cyclodextrin Glycosyltransferase

CGTase from *Paenibacillus* sp. RB01 was purified by starch adsorption technique prior to be used as a biocatalyst in the transglycosylation reaction for the synthesis of alkyl glycosides [7]. The reaction mixture containing 0.6% (w/v) β-CD and different alcohols as acceptors was incubated with CGTase (200 U/mL) in 50 mM acetate buffer pH 6.0 at 40 ℃ for 24 hours with continuous shaking. The effect of alkyl chain length and concentration on CGTase were carried out using phenolphthalein method [8]. The relative coupling activity was calculated from the disappearance of β-CD substrate. The decrease in absorbance at 550 nm was measured and conversion of $\Delta A550$ to μmoles of β-CD was quantitated using β-CD phenolphthalein calibration curve. The β-CD disappearance in buffer was set as 100 percent.

2.3. TLC Analysis

The reaction mixture was evaporated at 30 ℃ for 3 hours. The evaporated sample was adjusted to 100 μL with distilled water. The product were analyzed by applying 5 μL of each sample on siliga gel 60 F_{254} aluminium sheets with ethyl acetate: acetic acid: water (3:1:1) as a mobile phase. The compounds were visualized by spraying with sulfuric: methanol (1:2 by vol) and heating at 110 ℃ for 10 minutes. The amount of product was determined from spot intensities using GeneTools program of SYNGENE Synoptic Ltd., England.

2.4. Preliminary Characterization of Glycoside Product Structure

Alkyl glycoside from the CGTase-catalyzed reaction was further hydrolyzed by hydrolytic enzyme to confirm that the product was glycoside derivative. The reaction mixture was evaporated to remove the alcohol substrate before treatment with glucoamylase (final concentration of 40 U/mL) for 1 hour. The reaction was stopped by boiling at 100 ℃ for 10 minutes. The alpha linkage between alcohol and glucose residue was also checked by treatment the transglycosylation reaction with α-glucosidase (final concentration of 40 U/mL) at 40 ℃ for 3 hours. The result obtained was checked with TLC.

2.5. Donor Specificity

To find an appropriate donor, various carbohydrates including β-CD, raw starch, and soluble starch both from potato and cassava - flomax® were used as a glucosyl donor. Various donors were used at the final

concentration of 0.6% (w/v) under the optimal condition for CGTase as described previously. Alkyl glycoside products produced from each donor were quantitated from spot intensities on the TLC plate.

3. Results and Discussion

3.1. The Effect of Alcohol Length and Concentration on the Production of Alkyl Glycosides

The effect of alcohols on coupling activity of CGTase was investigated in buffer and in a mixture of buffer and alcohol solution with varying alcohol length and concentration at the optimum pH (6.0) of the enzyme for 24 hours.
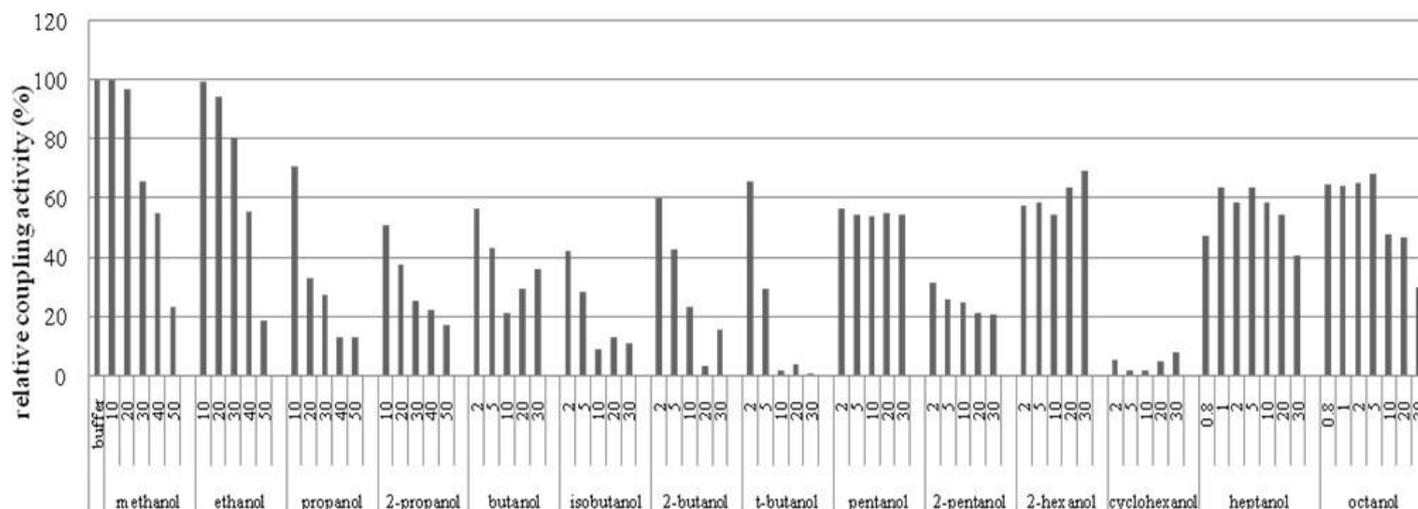

Fig. 1: Relative coupling activity of CGTase in alcohol solvent mixtures. Residual activity (%) was relative to the coupling activity of CGTase incubated with 0.6% (w/v) β-CD and various concentration of alcohol in 50 mM acetate buffer solution (pH 6.0) at 40 ℃ for 24 hours. All values were average from two separate experiments.

As can be seen in Fig. 1, when the concentration of alcohol increased, the activity of the enzyme was dramatically decreased. Medium and long chain alcohols at the concentration above 5% (v/v) are non soluble so shaking is needed and might cause the reaction to proceed non-uniformly. Thus, the activity of CGTase in biphasic system still remained whereas the coupling activity of the enzyme in monophasic system dramatically decreased. These results showed that the alcohol length and concentration had significant effect on CGTase activity. Cyclohexanol has been shown to compete with phenolphthalein to form complexation with hydrophobic cavity of CD. Hence, the complexation between phenolphthalein and CD appeared to be strongly effected [9]. Although branched chain primary alcohols had more effect on enzyme activity but they were more soluble than that of normal chain primary alcohol so the production yield was found to be higher. From these results, isobutanol was chosen as an appropriate acceptor. The TLC chromatogram of the CGTase catalyzed the transfer of glucose from β-CD to various alcohols are shown in Fig. 2. By adsorption chromatographic mechanism, the expected alkyl glycoside products with less polarity due to the length of alkyl chains migrated with larger R_f values than those of sugar in the non-polar mobile phase. The number of spots detected was dependent on polarity of the products which correlated with the length as well as the conformation of alkyl chain and the number of glucose residues. The product obtained from the CGTase-catalyzed transglycosylation reaction was more diversified than those from action of β-glucosidase [10].

3.2. Preliminary Characterization of Reaction Products Using Amylolytic Enzymes

Reaction mixture of selected acceptor was further hydrolyzed with glucoamylase–an enzyme which hydrolyzes glycosidic linkage between glucose residues and α-glucosidase to confirm the linkage between alcohol acceptor and glucose. Fig. 3 showed that after treatment with glucoamylase the amount of hydrolysis product disappeared, only the spot of glucose and isobutyl monoglucoside increased due to the glycosidic linkage was hydrolyzed except those between alcohol and glucose. The reaction mixture was further treated with α-glucosidase to elucidate the linkage between alcohol and glucose. It can be been that the spot of

isobutyl glucoside disappeared (Fig. 3, Lane 8) which could be due to the α-linkage was hydrolyzed. Thus, it seems likely that CGTase could catalyze transglycosylation reaction from β-CD to alcohols.

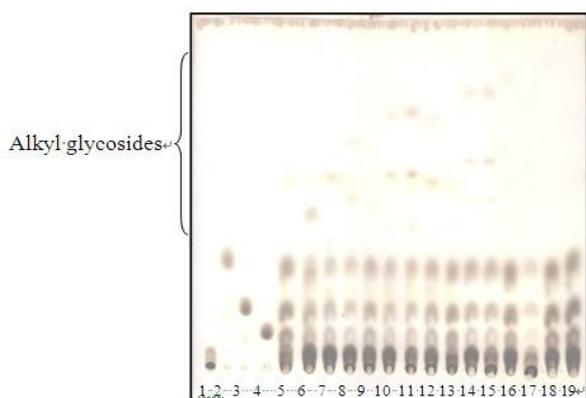

Fig. 2: TLC analysis of the transglycosylation products from β-CD to various alcohols by CGTase. Lane 1-4 standard β-CD, glucose, maltose and maltotrios, Lane 5 reaction mixture without alcohol acceptor, Lane 6-7 reaction mixture with 30% (v/v) methanol and ethanol, Lane 8-13 reaction mixture with 10% (v/v) 1-propanol, 2-propanol, 1-butanol, isobutanol, 2-butanol and tert-butanol, Lane 14-15 reaction mixture with 5% (v/v) pentanol and 2-pentanol, Lane 16-17 reaction mixture with 2% (v/v) 2-hexanol and cyclohexanol, Lane 18-19 reaction mixture with 1% (v/v) 1-heptanol and 1-octanol.
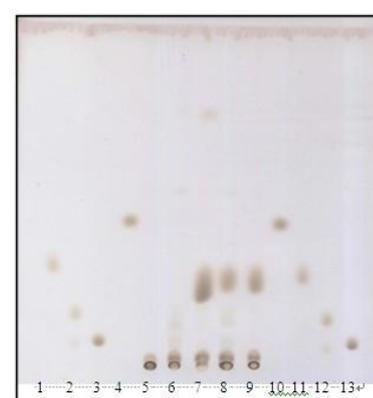
Fig. 3: TLC chromatogram of amylolytic enzyme treatment of CGTase reaction products. Lane 1-4 standard glucose, maltose, maltotriose and methyl monoglucoside Lane 5 reaction mixture at 0 minute incubation, Lane 6 reaction mixture after 24 hours incubation, Lane 7 reaction mixture (24 hours) treated with glucoamylase, Lane 8 reaction mixture (24 hours) with α-glucosidase and Lane 9 reaction mixture (24 hours) with glucoamylase and α-glucosidase, Lane 10-13 standard methyl monoglucoside, glucose, maltose and maltotriose.

3.3. Donor Specificity

CGTase could use various types of carbohydrate sources as a glycosyl donor. The products obtained from all donors were comparable as shown in Table1. Raw starch was successfully used as a glycosyl donor with similarity in product types and the amount obtained. Thus it might have high potential for the application in the manufacturing process because it is inexpensive and could be easily obtained from renewable agricultural resource.

Table 1: The amount of alkyl glycoside products from various glycosyl donors

Donors	Total production yield from various donors (mol %)*			
	Isobutyl monoglucoside	Isobutyl maltoside	Isobutyl maltotrioside	Total
β-CD	26.4	21.9	11.8	60.2
Soluble stach	24.6	21.9	10.0	56.5
Flomax®	26.4	21.9	10.0	58.3
Raw starch	22.8	18.2	10.0	51.0

*mole % represent the mole percent of alkyl glycosides spot relative to the total mole present in the β-CD substrate (0.6% w/v) each values were from separate experiment and quantitated using methyl-α-D-glucopyranolside as standard.

5. References

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