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Effects of substrates and reaction conditions on production of cyclodextrins using cyclodextrin glucanotransferase from newly isolated *Bacillus agaradhaerens* KSU-A11

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Abstract The effects of reaction conditions on cyclodextrins (CDs) production by CGTase from newly isolated *Bacillus agaradhaerens* KSU-A11 is reported. Among six types of starch tested, potato starch gave highest starch conversion into CDs. In addition, CDs yield was about three fold higher when using gelatinized potato starch in comparison to raw starch. The total CDs production was increased with increasing pH, showing maximum starch conversion at pH 10. Furthermore, the proportion of γ-CD was relatively higher under slightly acidic-neutral conditions than at alkaline pH with a maximum proportion of 35.6% at pH 7 compared to 7.6% at pH 10. Maximum starch conversion into CDs was seen at reaction temperature of 55ºC. Lower reaction temperature led to higher proportion of γ-CD with maximum percentage at 35ºC. Cyclization reaction was significantly promoted in the presence $CaCl_2$ (10 mM), while in the presence of ethyl alcohol there was significant decrease in CD production particularly at high concentration. β-CD was the major product up to 1 hr reaction period with traces of α-CD and no detectable γ-CD. However, as the reaction proceed, γ-CD started to be synthesised and α-CD concentration increased up to 4 hrs, where the CDs ratios were 0.27:0.65:0.07 for α-CD:β-CD:γ-CD, respectively. In addition, optimum CGTase/starch ratio was obtained at 80 U/g starch, showing highest starch conversion into CDs. All the parameters involved have been shown to affect the products yield and/or specificity of *B. agaradhaerens* KSU-A11 CGTase.

Keywords: *Bacillus agaradhaerens*, cyclodextrin glucanotransferase, cyclodextrinc, enzymatic reaction, starch

INTRODUCTION

Cyclodextrins (CDs) are non-reducing, cyclic oligosaccharides composed of D-glucose units linked by α-1,4 glycosidic bonds. CDs are doughnut-shaped molecules with a hydrophilic outer surface and a relatively hydrophobic cavity (Del Valle, 2009). With such structural features, they are able to form complexes with molecules, or part of them, especially hydrophobic residues, changing the physicochemical properties, such as solubility and stability of the guest compounds (Rahman et al. 2006; Szerman et al. 2007). This property has been used to stabilize and solubilise various substances of interest in numerous applications in the pharmaceutical, cosmetics, and food and textile industries, in addition to separation of enantiomers to extract toxic chemicals from waste streams and in soil bioremediation (Fava and Ciccotosto, 2002; Li et al. 2007; Astray et al. 2009; Del Valle, 2009).

CDs are formed by the action of cyclodextrin glucanotransferase (CGTases) (EC 2.4.1.19). CGTases catalyze the formation of CDs from starch and related α-1 → 4 linked glucose polymers via a transglycosylation reaction (Tonkova, 1998; Leemhuis et al. 2010). Several species of *Bacillus* are the major sources of CGTase, in addition to some species of *Klebsiella*, *Thermococcus* sp., *Micrococcus*

luteus and other microorganisms (Horikoshi, 1999; Tachibana et al. 1999; Biwer et al. 2002; Charoensakdi et al. 2007; Szerman et al. 2007). As the separation of different CDs is costly and time-consuming, CGTases that synthesise predominantly one type of CD are of great interest (Li et al. 2007; Otero-Espinar et al. 2010). Depending on the source of organism, CGTases often produce varying levels of α-, β- and γ-CD. However, most CGTases from alkaliphilic bacteria convert starch into β-CD as a predominant product; although a mixture of other CD forms are still produced in varying ratios (Horikoshi, 1999; Atanasova et al. 2011). There are different approaches to enrich the yield of the target type of CD, including search for novel CGTase for this purpose, enzyme engineering of CGTase at central active site cleft (Takada et al. 2003; Goh et al. 2007) and, alternatively, modifying the substrates and enzymatic reaction conditions. Such parameters include starch source and concentration (Cheirsilp et al. 2010), reaction pH (Hirano et al. 2005), presence of certain additives and precipitants (Martins and Hatti-Kaul, 2003). In this study, we report the influences of different reaction parameters on the ratio and total yield of CDs produced by the action of CGTase from a newly isolated alkaliphilic *Bacillus agaradhaerens* KSU-A11 (Ibrahim et al. 2010).

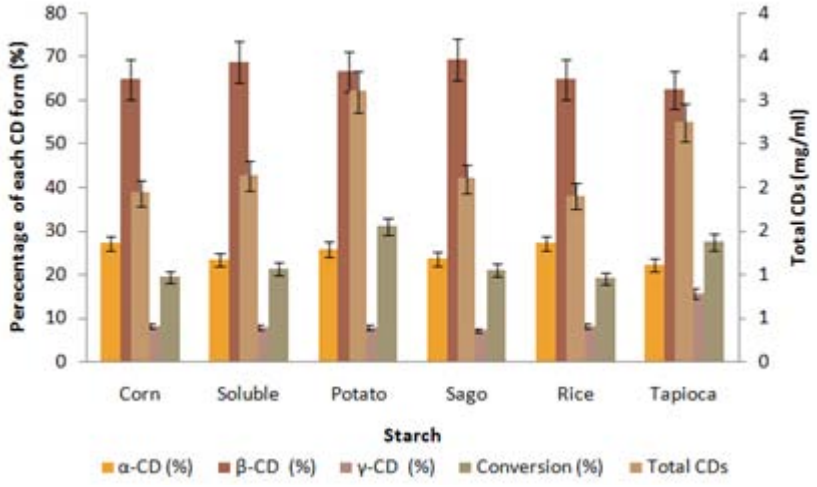

Fig. 1 Effect starch types on CDs production by *B. agaradhaerens* KSU-A11. The reaction mixtures containing 1% (w/v) starch and 0.1 U/ml of CGTase in 50 mM glycine buffer, pH 9, were incubated at 50ºC for 3 hrs. Production of CDs was determined by HPLC analysis. Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

MATERIALS AND METHODS

Cyclodextrins (α-, β-, and γ-CD), (G2–G7), phenolphthalein, glucoamylase (A-7420), maltodextrin, and other chemicals of analytical grade were purchased from Sigma (St. Louis, MO, USA). Different starch types were purchased from Merck (Darmstadt, Germany). The tapioca starch used was of food grade and sourced from a local manufacturer.

Bacterial stain, growth conditions, and partial purification of CGTase

Previously isolated alkaliphilic *B. agaradhaerens* KSU-A11 was cultured in an alkaline medium (pH 10.2) containing soluble starch as substrate, and the enzyme was partially purified from the culture supernatant by adsorption to corn starch as described earlier (Ibrahim et al. 2010). Briefly, corn starch and ammonium sulphate were added to cell-free supernatant to a concentration of 5% (w/v) and 1 M, respectively, and was kept at 4ºC with constant moderate mixing to allow CGTase adsorption. The mixture was then centrifuged at low speed (1500 x g) and the pellet was collected and washed with cold 1 M ammonium sulphate solution to remove unbound proteins. Adsorbed CGTase was eluted from the corn starch by incubating the pellet for 30 min at 37ºC in small volume of 50 mM Tris-HCl buffer, pH 8.0, containing 1 mM β-CD. The eluate (280 ml) was concentrated using an Amicon

ultrafiltration membrane kit (10 kDa cut-off membrane) and then dialyzed against 50 mM Tris-HCl buffer overnight at 4ºC and stored at -20ºC till use. The partially purified CGTase had the specific activity of about 64.5 µmol/min. mg^{-1}.

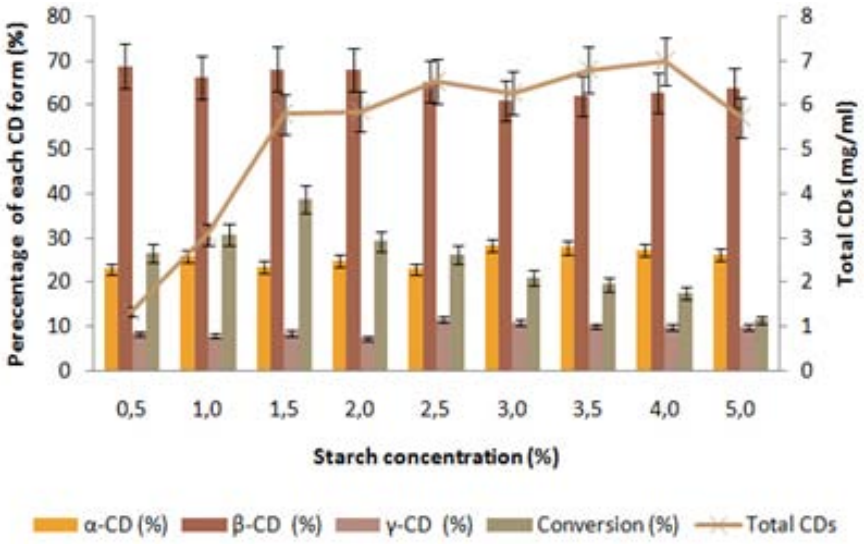

Fig. 2 Effect of concentration of potato starch on CDs production by *B. agaradhaerens* KSU-A11. The reaction mixtures containing potato starch (0.5-5%, w/v) and 0.1 U/ml of CGTase in 50 mM glycine buffer, pH 9, were incubated at 50ºC for 3 hrs. Production of CDs was determined by HPLC analysis. Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

Enzyme assay

CGTase activity was measured as β-CD forming activity (Thiemann et al. 2004). 750 µl of a 1% (w/v) starch solution prepared in 50 mM glycine-NaOH buffer, pH 9, was pre-incubated at 50ºC for 5 min. Then, 25 µl of enzyme sample was added and after incubating for 20 min at 50ºC, the reaction was quenched by adding 375 µl of 0.15 M NaOH. Subsequently, 100 µl of 0.02% (w/v) phenolphthalein prepared in 5 mM Na_2CO_3 was added and after standing at room temperature for 15 min, the colour intensity was measured at 550 nm. Reaction mixture containing inactive enzyme was used as a control. One unit of CGTase activity was defined as the amount of enzyme releasing one µmol of β-CD per min under the defined assay conditions. A calibration curve was made using different concentration of β-CD in 50 mM glycine-NaOH buffer, pH 9. Protein content was measured according to Bradford (1976) using bovine serum albumin as standard protein.

Analysis of cyclodextrins by HPLC

For quantitative analysis of CDs, the enzymatic reactions were carried out and then stopped by placing the samples in a boiling water bath for 5 min. The produced linear oligosaccharides were initially hydrolyzed by glucoamylase. For this, the starch hydrolysate were cooled and 50 µl was mixed with 5 µl (2 U) of glucoamylase and 45 µl 0.4 M sodium acetate buffer, pH 5, and incubated for 1 hr at 40ºC. Then, the reaction was stopped by placing the samples in a boiling water bath for 5 min and the mixtures were filtered through a 0.45 µm membrane filter. The concentrations of different CDs produced were determined by HPLC analysis under the following conditions: column, Aminex HPX-42A (Bio-Rad); mobile phase, filtered distilled water; flow rate, 0.6 ml/min; column temperature, internal 80ºC; external 55ºC; refractive index detector (La Chrom, L7490 Merck-Hitachi, Ltd. Tokyo, Japan). Calibration curve was established using α-, β- and γ-CD (1-10 mM). The percentage of starch conversion (%) was defined as the weight percentage of initial substrate converted into total CDs (g CD/100 g starch). All reactions and analysis were carried out in triplicate.

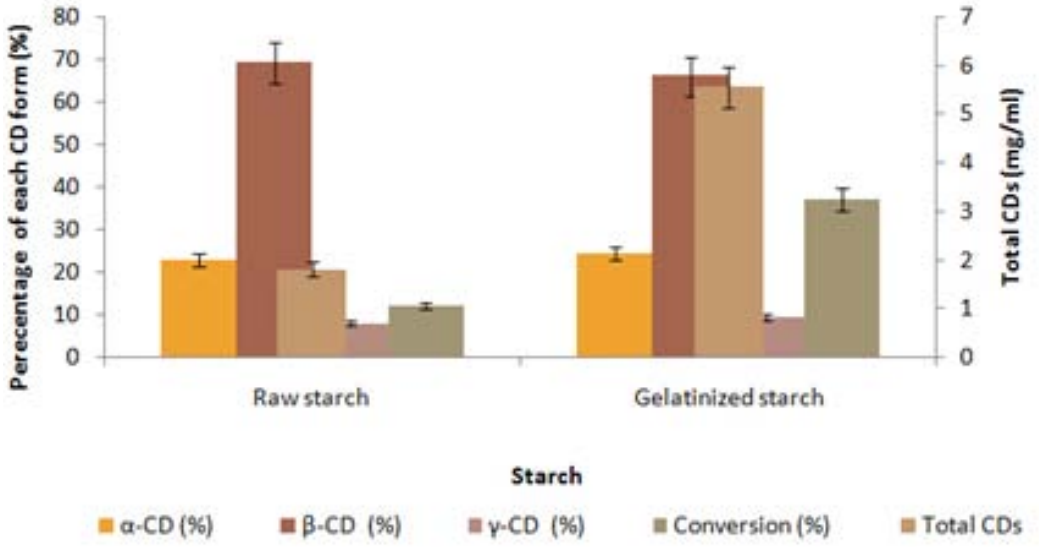

Fig. 3 CDs production by *B. agaradhaerens* KSU-A11 using raw and gelatinized corn starch. The reaction mixtures containing 1.5% (v/w) potato starch and 0.1 U/ml of CGTase in 50 mM glycine buffer, pH 9, were incubated at 50ºC for 3 hrs. Production of CDs was determined by HPLC analysis. Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

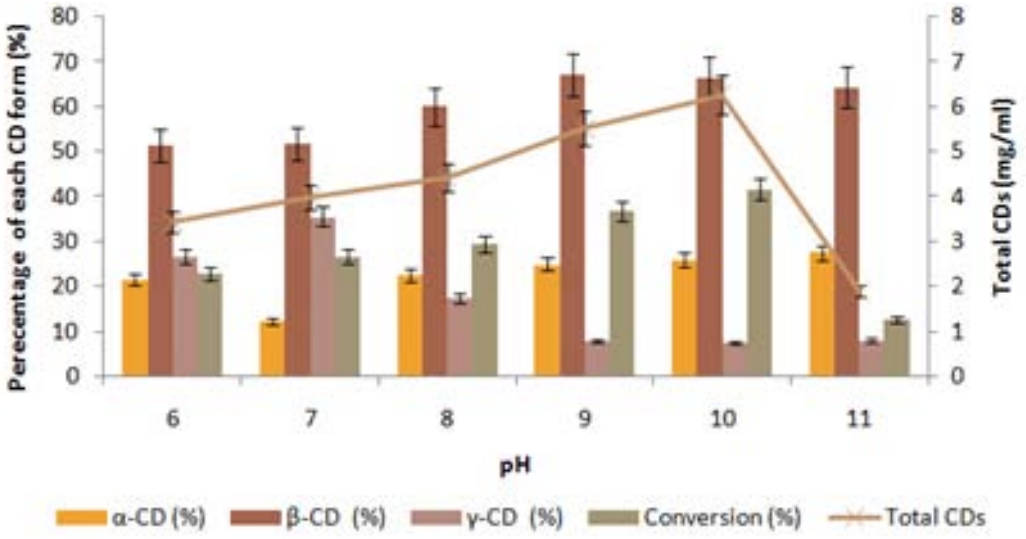

Fig. 4 Effect of reaction pH on CDs production by *B. agaradhaerens* KSU-A11. The reaction mixtures containing 1.5% (v/w) gelatinized potato starch and 0.1 U/ml of CGTase in different buffers (pH 6-11), were incubated at 50ºC for 3 hrs. Production of CDs was determined by HPLC analysis. Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

Effect of different substrates on CDs production

For investigation of the effect of substrates on CDs formation, different substrates including corn, soluble, potato, sago, rice and tapioca starch, were boiled for 10 min in 50 mM glycine buffer (pH 9), and cooled to room temperature. Then, partially purified *B. agaradhaerens* KSU-A11 CGTase was reacted with 1 ml of 1% (w/v) substrate in glycine-NaOH buffer, pH 9 and 50ºC for 3 hrs. Enzymatic reactions were stopped by boiling for 10 min, and the CDs produced were analyzed using HPLC analysis as described above. Furthermore, the effect varying concentration of the best starch source, ranged from 0.5 to 5%, on the formation of CDs, were studied.

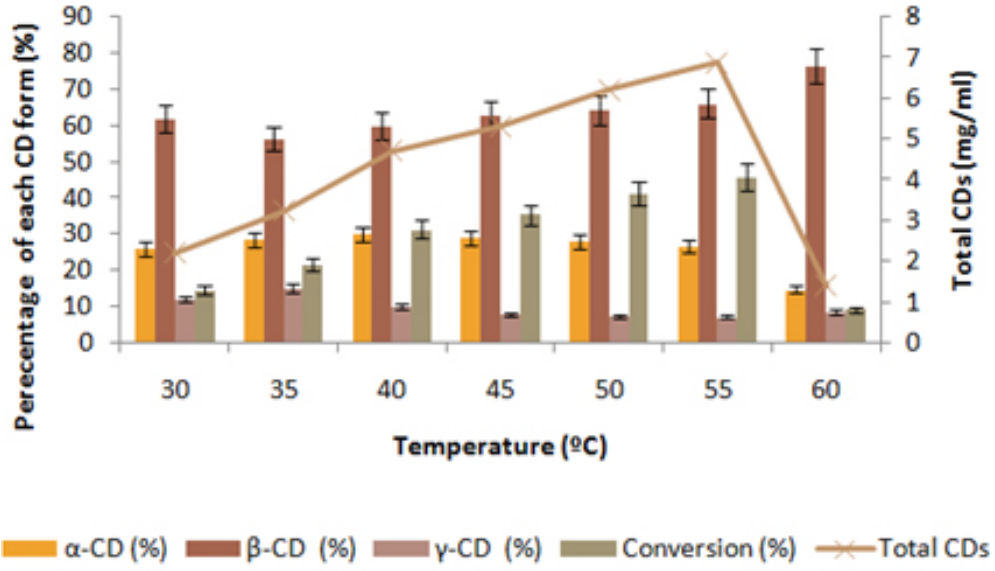

Fig. 5 Effect of reaction temperature on CDs production by *B. agaradhaerens* KSU-A11. The reaction mixtures containing 1.5% (v/w) gelatinized potato starch and 0.1 U/ml of CGTase in glycine buffer, pH 10, were incubated at various temperature (30-60ºC) for 3 hrs. Production of CDs was determined by HPLC analysis. Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

Effects of raw and gelatinized starch on CDs production

Gelatinized starch was prepared by heat treatment of 1.5% (w/v) potato starch, suspended in 50 mM glycine buffer of pH 9, in a boiling water bath for 15 min. Raw and gelatinized potato starch were used as a substrates. Enzymatic reactions and CDs analysis were carried out as described earlier.

Effects of pH and temperature on CDs production

Partially purified *B. agaradhaerens* KSU-A11CGTase was reacted with 1.5% gelatinized potato starch prepared in 50 mM buffers ranging from pH 6 to 11 for 3 hrs at 50ºC. Different suitable buffers were used including 50 mM sodium acetate (pH 6.0), 50 mM sodium phosphate (pH 7-8), 50 mM glycine-NaOH buffer (pH 9.0-10) and 50 mM carbonate-bicarbonate buffer (pH 10-11), respectively. The effect of reaction temperature on CDs production was studied by carrying out the enzymatic reaction at temperature range of 30 to 60ºC at optimum pH for 3 hrs and CDs analysis were carried out.

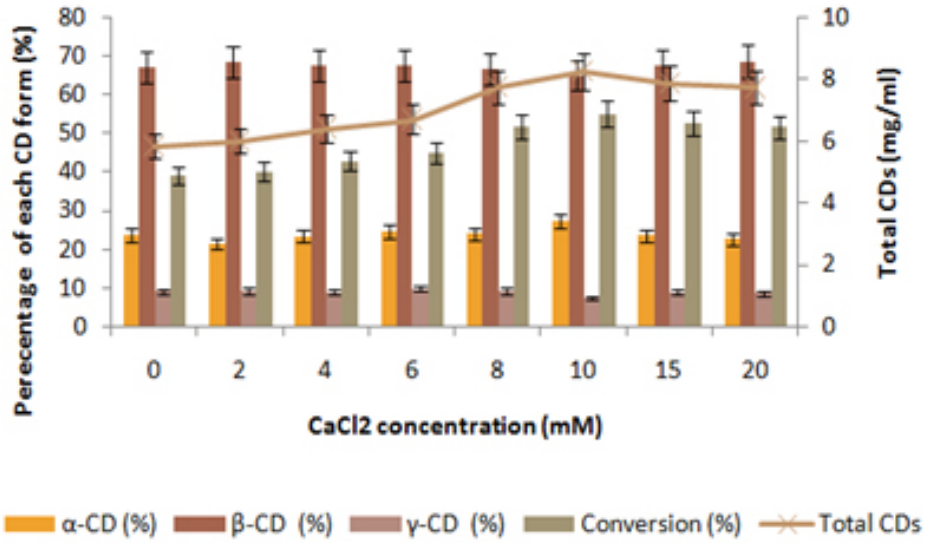

Fig. 6 Effect of $CaCl_2$ on CD production by *B. agaradhaerens* KSU-A11. The reaction mixtures containing 1.5% (v/w) gelatinized potato starch and 0.1 U/ml of CGTase in glycine buffer, pH 10, containing various concentration of $CaCl_2$ (0-20 mM) were incubated at 50ºC for 3 hrs. Production of CDs was determined by HPLC analysis Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

Effects of ethyl alcohol on CDs production

Partially purified CGTase was reacted with 1 ml of 1.5% gelatinized potato starch in 50 mM glycine buffer, pH 10 in the presence of ethyl alcohol (0-30%). Ethyl alcoholwas only added once at the beginning of the reaction.

Time course of CDs production and enzyme/substrate ratio

Partially purified CGTase (200 µl) was added to 3.8 ml of 1.5% (w/v) gelatinized potato starch prepared in 50 mM glycine-NaOH buffer, pH 9, and incubated at 50ºC. Aliquots (200 µl) of the reaction mixture were withdrawn at different time intervals up to 8 hrs and the reaction was stopped by placing in a boiling water bath for 5 min. CDs were analyzed by HPLC as described above. In addition, optimum enzyme/starch ratio was determined by performing the enzymatic reaction using various amounts of CGTase (20 to 200 U/g starch) at the optimum reaction conditions.

RESULTS AND DISCUSSION

Effect of substrates on CDs production

The influence of various substrates including corn, soluble, potato, sago, rice and tapioca starch, in 50 mM glycine, pH 9 buffers were studied to identify the best substrate for CDs production, using partially purified *B. agaradhaerens* KSU-A11 CGTase. The ratio and total CDs yield were determined using HPLC analysis. In this study, among the used starch, potato starch appeared to be the best substrate for the enzyme, producing 3.1 mg CDs from 10 mg starch, followed by tapioca starch (2.7 mg), while corn and rice starch showed the lowest CDs yield (1.9 mg), (Figure 1). The best starch source for CDs production varied according to the enzyme source, that tapioca starch showed highest CDs production by CGTase from *Bacillus* sp. G1 (Sian et al. 2005) and from *Bacillus* sp. C26 (Cheirsilp et al. 2010), while soluble starch was the best substrate for CGTase from *Klebsiella pneumoniae* AS-22 (Gawande and Patkar, 2001). This suggested that the various sources of starch could affect the CDs production, which is probably caused by the differences in the starch granules structure and properties (Sian et al.

2005; Goh et al. 2007). However, there was no significant difference in the ratios of different CDs by using different starch, with β-CD as the predominant product (66.4%). In addition, the effect of different concentrations of potato starch on CDs production was investigated (Figure 2). The highest conversion (38.7%) of potato starch into CDs was obtained at concentration of 1.5% (w/v). At higher starch concentration, there was an increase in the total amount of the produced CDs; however, there was significant decrease in the conversion rate (Figure 2). However, the varying starch concentrations had no significant effect on the ratio of different produced CDs. This result is relatively similar to that previously reported for CGTases from *Bacillus megaterium* and *Bacillus* sp. G1 where the optimum starch concentration for CDs production was 1.5% and 2% respectively (Goh et al. 2007; Zhekova et al. 2008). The lower conversion degree of the higher starch amount may be due to CGTase inhibition by CDs. Decrease in CDs yield, when high starch concentrations are applied, has been reported by other authors (Yamamoto et al. 2000; Sian et al. 2005). The enzyme transformation of the soluble starch is impeded not only by the inhibitory action of CDs, but also by the high dextrose equivalent (Pishtiyski and Zhekova, 2006; Zhekova et al. 2008). Furthermore, increasing viscosity of the starch slurry at higher concentrations is a common problem in the starch industry, and therefore starch is usually liquefied using high temperature or amylases (Pedersen et al. 1995, Goh et al. 2007).

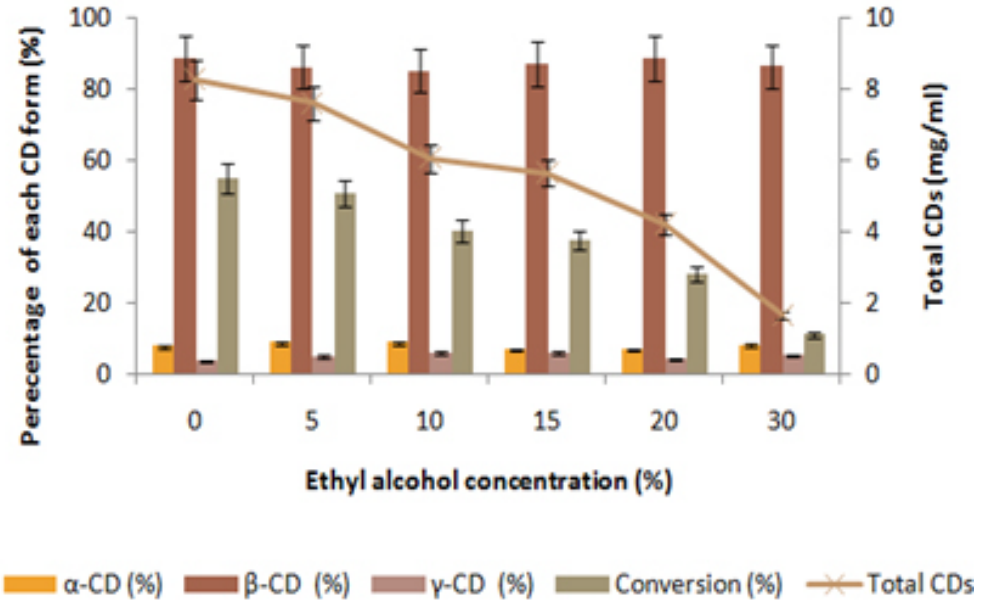

Fig. 7 Effect of ethanol on CDs production by *B. agaradhaerens* KSU-A11. The reaction mixtures containing 1.5% (v/w) gelatinized potato starch and 0.1 U/ml of CGTase in glycine buffer, pH 10, containing various concentration of ethanol (0-30%) were incubated at 50ºC for 3 hrs. Production of CDs was determined by HPLC analysis. Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

Effects of raw and gelatinized starch on CD production

Since 1.5% potato starch gave highest conversion (38.7%) into CDs, it was used in all the following experiments. Comparison between raw and gelatinized potato starch as a substrate for *B. agaradhaerens* KSU-A11 CGTase indicated that the total amount of produced CDs (5.6 mg) was about three fold higher when gelatinized starch was used compared to raw starch (1.82 mg), (Figure 3). However, there was no significant difference in the ratio of different forms of CDs. Raw starch is compact crystalline structure that is difficult to be degraded by starch degrading enzymes owing to the weak interaction of CGTase with raw potato starch granules (Tester et al. 2004). By gelatinization, the crystalline structure of starch is disrupted by heating in the presence of water. Gelatinized starch swells irreversibly creating larger surface/volume ratio for enzymatic reaction (Tester et al. 2004; Goh et al. 2007; Ratnayake and Jackson, 2007).

Effect of pH on CD production

Figure 4 shows the effect of pH of the reaction mixture on CDs production by action of *B. agaradhaerens* KSU-A11 CGTase on 1.5% gelatinized potato starch at 50ºC. As shown in Figure 4, total CDs production was increased with increasing pH, showing maximum CDs yield and starch conversion at pH 10 of 6.3 mg and 41.7%, respectively, with β-CD as the predominant CD type (66.4%). Interestingly, percentage of γ-CD was relatively higher under slightly acidic-neutral conditions (pH 6-7) than at alkaline pH with a maximum percentage of 35.6% at pH 7 compared to 7.6% at pH 10, at which maximum CDs yield was obtained. These results were in contrast to the result of CGTase from *B. agaradhaerens* LS-3C reported by Martins and Hatti-Kaul, (2003). In that study maximum conversion was obtained at pH 8, in addition at pH 10, α-CD was replaced by γ-CD which was undetectable in other pHs < 10. The size of the CDs (α-, β- or γ-CD) formed by a CGTase enzyme is determined by the number of glucose units (6, 7 or 8, respectively) that can be accommodated in the active site groove adjacent to the substrate cleavage site (van der Veen et al. 2000a; van der Veen et al. 2000b). The notable change in product profile with pH can thus be related to changes in the ionization state of the amino acids involved in the hydrogen bonding between the substrate and the sugar binding sites of the enzyme, which may promote accommodation of an additional glucose unit and hence formation of bigger CDs (Martins and Hatti-Kaul, 2003; Hirano et al. 2005).

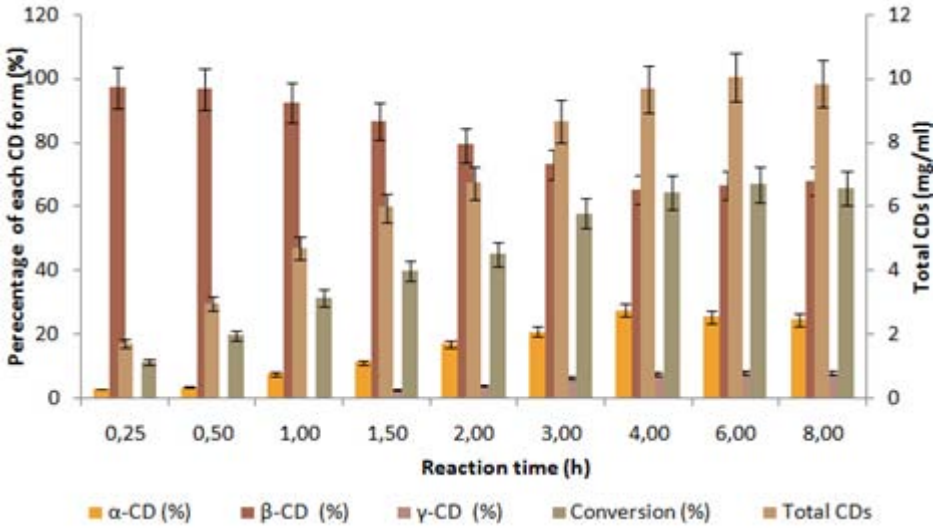

Fig. 8 Effect of reaction time on CDs production by *B. agaradhaerens* KSU-A11. The reaction mixtures containing 1.5% (v/w) gelatinized potato starch and 0.1 U/ml of CGTase in glycine buffer, pH 10. Aliquots (200 µl) of the reaction mixture were withdrawn at different time intervals up to 8 hrs and the reaction was stopped by placing in a boiling water bath for 5 min. Production of CDs was determined by HPLC analysis. Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

Effect of temperature on CDs production

The influence of reaction temperature on CDs production was studied by varying the reaction temperature from 30ºC to 60ºC. There was increase in the CDs yield and starch conversion with increasing temperature, with maximum value of 6.9 mg and 45.9% at 55ºC, respectively. However, the total CDs yield was drastically declined at 60ºC (Figure 5). Interesting, percentage of γ-CD was higher at lower temperature than increased temperature with maximum percentage (15.2%) at 35ºC, although the total CDs yield (3.2 mg) was much less in comparison to the maximum yield obtained at 55ºC (6.9 mg). The enzymatic reaction is enhanced by temperature due to the higher kinetic energy, however, according to the thermostability of the enzyme, higher temperature leads to enzyme denaturation and loose of function in a short period of time resulting in lower yield of the product (Kitcha et al. 2008; Cheirsilp et al. 2010).

Effect of $CaCl_2$ on CDs production

Further investigation of CDs production by the *B. agaradhaerens* KSU-A11 CGTase during 3 hrs reaction in the presence of 0-20 mM $CaCl_2$ in glycine buffer (pH 10) showed a gradual increase in CDs production level with increasing $CaCl_2$ concentration up to 10 mM to about 1.4-fold (8.3 mg) and starch conversion of 55.1% (Figure 6). However, $CaCl_2$ had no significant effect on the ratio of different CD forms. This result is in contrast to that previously reported by Martins and Hatti-Kaul, (2003), where production level of β-CD increased to about 1.5-fold with increasing $CaCl_2$ concentration up to 14 mM, indicating the difference in the CGTases properties of the two bacterial strains. Stimulation of the cyclization activity by $CaCl_2$ has been commonly observed for CGTases and could be to some extent due to increased enzyme stability, making it more effective for a long period of time (Gastón et al. 2009; Moriwaki et al. 2009).

Effect of ethyl alcohol on CDs production

Figure 7 shows overall CDs yield and ratio in the presence of ethyl alcohol (0-30%). The results indicated that ethyl alcohol has inhibitory effect on the CDs production particularly at higher concentrations. There are some reports about increase in the overall yield of CDs and/or alteration in the product specificity by performing the CGTase reaction in the presence of organic solvents (Blackwood and Bucke, 2000). However, there are other reports of inhibition of CDs production by ethyl alcohol (Martins and Hatti-Kaul, 2003; Goh et al. 2007). The reduction of CDs formation in the presence of ethyl alcohol is probably due to the denaturation of *B. agaradhaerens* KSU-A11 CGTase.

Time course of CDs production

CDs yield and ratio were analyzed up to 8 hrs of reaction period. As shown in Figure 8, the overall CDs yield increased with increasing reaction time and reached maximum yield and starch conversion of 9.7 mg and 64.4% after 4 hrs, respectively. β-CD was the major product up to 1 h reaction period with traces of α-CD and no detectable γ-CD. However, as the reaction proceed, γ-CD start to synthesised and α-CD concentration increased up to 4 h where the ratio of each CD form appeared to be constant with ratio of α-CD:β-CD:γ-CD of 0.27:0.65:0.07, respectively.

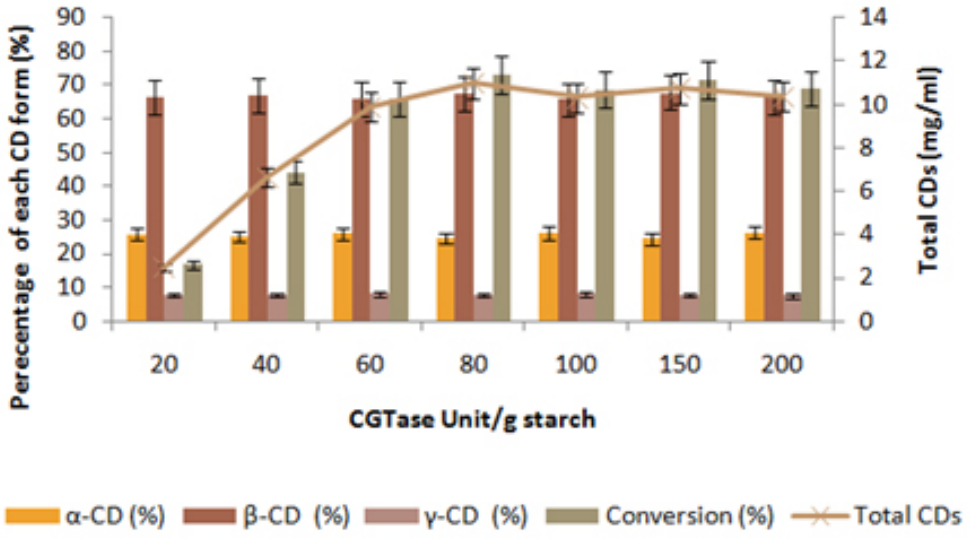

Fig. 9 Effect of enzyme concentration on CDs production by *B. agaradhaerens* KSU-A11. The reaction mixtures containing 1.5% (v/w) gelatinized potato starch and CGTase (20-200 U/ g starch) in glycine buffer, pH 10, were incubated at 50ºC for 4 hrs Production of CDs was determined by HPLC analysis. Conversion (%): g CD/100 g starch; left y axis: percentage of each CD form; right y axis: Total CDs yield. Standard deviations are shown as bars.

Effect of amount of enzyme/substrate on CDs synthesis

In order to determine the optimum amount of CGTase, the production of β-CD from 1.5% potato starch with different amounts of enzyme was investigated (Figure 9). The overall CDs yield increased with increasing amounts of enzyme. The optimum amount of enzyme to starch was found to be 80 U/g potato starch since above this ratio there was no further increase in CDs synthesis production. In contrast, with increasing enzyme concentration of CGTase from *B. agaradhaerens* LS-3C the β-CD production decreased drastically and became undetectable at 30 U/g starch (Martins and Hatti-Kaul, 2002). In that case, it was concluded that because the CGTase from *B. agaradhaerens* LS-3C exhibited a low rate of CD production and the competing hydrolysis reactions of CD seemed to dominate at high concentrations of the enzyme. In addition, this ratio (80 U/g starch) is higher than the optimum ratio reported for β-CD production by CGTase from *Bacillus* sp. C26, where the optimum value was 48/g tapioca starch, (Cheirsilp et al. 2010), and 50 U/g-starch for β-CD production by a CGTase from *Bacillus circulans* TISTR 907 (Charoenlap et al. 2004)

CONCLUDING REMARKS

In this study, the effects of reaction conditions on cyclodextrins (CDs) production by CGTase from newly isolated *Bacillus agaradhaerens* KSU-A11 was investigated. Overall, optimization of the reaction conditions (1.5% gelatinized potato starch, pH 10, 55ºC, 10 mM $CaCl_2$, and 80 U/g potato starch) for CDs production by *B. agaradhaerens* KSU-A11 CGTase led to increase of starch conversion into CDs by about two fold. Furthermore, it was found that pH, temperature and reaction time had significant effect on the type of the produced CD (α-, β- and γ-CD). Some of the studied parameters were previously reported to have a beneficial influence on CDs production while contradictory results have been also reported in other publications. Therefore, it can be concluded that reaction condition studies are still essential for each case of interest. In addition, the results also indicated the possibility to shift the product specificity of the *B. agaradhaerens* KSU-A11 CGTase to enrich the yield of the target type of CD, by modifying the substrates and enzymatic reaction conditions, according to the required industrial application.

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