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Full Length Research Paper

Purification and characterization of a thermostable cyclodextrin glycosyltransferase from *Thermoanaerobacter* sp. P4

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Cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19) from a thermophilic anaerobic bacterium, *Thermoanaerobacter* sp. P4, was purified by ammonium sulfate precipitation followed by α-cyclodextrin epoxy activated-sepharose 6B column chromatography. Enzyme was purified 141 fold and had the specific activity of 143.8 U/mg proteins. Purification yields after ammonium sulfate precipitation and affinity chromatography were 25.8 and 17.8%, respectively. SDS-PAGE analysis showed that enzyme was purified successfully and had a single band. Molecular weight of the enzyme was determined as 68.7 kDa. The enzyme had optimum cyclization activity at 80 to 90°C and hydrolyzing activity at 90°C and maintained 87 and 95% of these activities at 95°C, respectively. Optimal pH was found as 7.0. It retained full activity at 80°C for 4 h. Enzyme was strongly inhibited by $HgSO_4$ and $AgNO_3$. Addition of 1 mM $CaCl_2$ increased the enzymatic activity up to 7%. This novel enzyme could be a good candidate for industrial applications according to its characteristic found in the current study.

Key words: Cyclodextrin glycosyltransferase, cyclodextrin production, *Thermoanaerobacter* sp. P4, thermophilic, enzyme purification, enzyme characterization.

INTRODUCTION

Cyclodextrin glycosyltransferase (CGTase, EC2.4.1.19) is a starch converting extracellular enzyme which belongs to α-amylase family (family 13) of glycoside hydrolases (Bertoldo and Atranikian, 2002; van der Maarel et al., 2002). It is a unique enzyme capable of producing cyclodextrins (CDs) through intramolecular transglycosylation reaction called cyclization (Ibrahim et al., 2005; Tonkova, 1998). Beside the cyclization reaction, this enzyme also catalyzes intermolecular transglycosylation reactions, namely, coupling reaction (opening of the CD ring and transferring the resulting linear oligosaccharides to acceptor molecules) and disproportionation reaction (transfer of linear oligosaccharides to acceptors) (Nakamura et al., 1994). CGTase also possesses weak hydrolyzing activity (Alcalde et al., 2003).

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CDs are cyclic oligosaccharides consisting of mainly six, seven and eight glucose units named as α, β and γ-CD, respectively, linked via α(1-4) glucosidic bonds (Biwer et al., 2002; van der Ween et al., 2000). Due to their hydrophobic cavity and hydrophilic outer surface, they are able to form inclusion (host-guest) complexes with a variety of organic and inorganic molecules, thus changing the chemical and physical properties of the guest molecules (Del Valle, 2004; Singh et al., 2002). Inclusion complex formation makes CDs attractive for the use in wide industrial applications including food, pharmacy, agriculture, cosmetics, analytical chemistry (Brewster and Loftsson, 2007; Del Valle, 2004; Loftsson and Duchene, 2007; Song et al., 2009; Szente and Szejtli, 2004).

CGTases are produced by various bacteria, mainly by mesophilic bacteria such as *Bacillus macerans* (Stavn and Granum, 1979), *Bacillus circulans* (Szerman et al., 2007), *Bacillus firmus* (Saverage et al., 2008), *Bacillus agaradhaerens* LS-3C (Martins and Hatti Kaul, 2003), *Paenibacillus pabuli* US132 (Jemli et al., 2007) *Klebsiella*

pneumoniae AS-22 (Gawande and Patkar, 2001) and *Brevibacterium* sp 9605 (Mori et al., 1994). In general, *B. macerans* is used in the commercial productions (Biwer et al., 2002). However industrial production of CDs require higher temperatures for the liquefaction of starch at which CGTases from mesophilic bacteria are not stable. It is performed by using thermostable α-amylases or by jet cooking before the addition of CGTases (Biwer et al., 2002; Jorgensen et al., 1997; Wind et al., 1995).

Some extremophilic CGTases from thermophilic anaerobic bacteria *Thermoanaerobacter* sp (Norman and Jorgensen, 1992) and *Thermoanaerobacterium* EM1 (Wind et al., 1995), archea *Thermococcus* sp. (Tachibana et al., 1999), *Thermocoocus kodakaraensis* (Rashid et al., 2002) and *Pyrococcus furiosus* (Lee et al., 2007) were isolated and characterized in the last two decades. These enzymes have optimal activities between 80 and 100°C and they also have higher hydrolyzing activities than the CGTases of mesophilic bacteria. Therefore production of CDs can be realized in one step with a single enzyme (Alcalde et al., 1999).

Interests in the usage of thermophilic enzymes are increasing due to their various advantages over mesophilic enzymes (Haki and Rakshit, 2003). Working with thermophilic enzymes at high temperatures reduces risk of contamination, improves reaction rate, lowers viscosity and increases solubility of substrates (Biwer et al., 2002). Researches are focused on the improvement of activity, selectivity, stability of these enzyme by site directed mutagenesis, chemical modification and immobilization of these enzymes as well as isolation and characterization of new enzymes (Alcalde et al., 2001; Haki and Rakshit, 2003; Cowan and Lafuente, 2011).

Although there are number of mesophilic CGTase producing strains characterized, CGTases from thermophilic bacteria are restricted. *Thermoanaerobacter* sp. P4 is thermophilic anaerobic bacterium which produces CGTase. It was identified and CGTase production conditions were determined in our previous study (Avci and Donmez, 2009). In this work, purification results and some properties of the purified CGTase from *Thermoanaerobacter* sp. P4 are demonstrated.

MATERIALS AND METHODS

CD standards were purchased from Sigma (USA) and soluble potato starch, yeast extract, peptone, ammonium sulfate and sodium dodecyl-sulphate were from Merck (Germany). Sepharose 6B was purchased from Amersham Biosciences (GE Healtcare, Sweden). Molecular weight kit for electrophoresis was purchased from Biorad (USA). 3,5-Dinitrosalicylic acid (DNS) was purchased from Sigma.

Bacterial strain

Thermophilic anaerobic bacterium was isolated from an oil field (Raman/Turkey) and identified as *Thermoanaerobacter* sp. P4 according to 16S rRNA studies and biochemical characteristics (Avci and Donmez, 2009). DSMZ medium no 144 was used for the growth of bacteria (www.dsmz.de/mediumlist). The medium was prepared under anaerobic conditions and dispensed to Hungate tubes or serum bottles with aluminum flacon under nitrogen atmosphere. Growth of the culture was carried out at 65°C for 24 h.

CGTase production

CGTase was produced under anaerobic conditions in 1 l serum bottles containing 750 ml medium. Medium contained soluble potato starch, 30 g/l; yeast extract, 5 g/L; casein, 5 g/l; $MgSO_4.7H_2O$, 1 g/l, K_2HPO_4 1.5 g/l with an initial pH of 7.5 to 8.0. After 30 h of growth at 65°C, bacterial cells were removed from the growth medium by centrifugation at 12000 x *g* for 15 min. Cell-free supernatant was used as the crude enzyme.

Enzyme purification

Ammonium sulfate precipitation and dialysis

Solid ammonium sulfate was slowly added to the crude enzyme solution until 85% of final concentration with continuous agitation for 8 h at 4°C and it was allowed to stand one night at 4°C to improve the precipitation efficiency. The precipitate was collected by centrifugation at 12000 x *g* for 15 min. It was dissolved in 10 mM Na acetate buffer pH 5.5 and dialyzed against the same buffer by using 10000 MWCO dialysis tube (Sigma, USA).

Affinity chromatography

Freeze dried epoxy-activated Sepharose 6B was used in the column. Affinity matrix was prepared using the method described by Sian et al. (2005). The gel was loaded into a 15 × 200 mm column (Pharmacia). Dialyzed enzyme solution was loaded onto column, and then it was flushed with 10 mM sodium acetate buffer pH 5.5 for 5 h at a flow rate of 36 ml/h. Elution of the bound CGTase was accomplished by using same buffer containing 1% α-CD. 3 ml fractions were collected by the help of the fraction collector (Spectra/Chrom CF-1) and each fraction was analyzed for CGTase activity and protein content.

Enzyme assay

Cyclization activity of CGTase was determined by the phenolphthalein method as described by Shim et al. (2004). The method is based on the decrease in colour intense due to inclusion complex formation of β-CD with phenolphthalein (Alcalde et al., 1999). 2% (w/v) soluble starch was used as substrate in 50 mM sodium acetate buffer pH 6.0. 0.1 ml of enzyme and 1 ml of soluble starch solution were incubated at 80°C for 10 min. The concentration of β-CD was determined by measuring the decrease in absorbance at 550 nm using a standard curve. One unit of enzyme activity was defined as the amount of enzyme producing 1 µmol of β-CD per minute. Unless otherwise indicated, enzyme activity or CGTase activity refers to cyclization activity in the text.

Hydrolytic activity was assayed by incubating 0.1 mL of enzyme solution with 0.3% (w/v) soluble starch in 50 mM sodium acetate buffer pH 6.0 for 20 min at 80°C. Then, the increase in reducing sugar content was measured by DNS method (Forouchi and Gunn, 1983). One unit of activity was defined as the amount of enzyme releasing 1 µmol of glucose per min under assay conditions.

Coupling activity was performed according to the method described by Nakamura et al. (1993) using α-, and β-CD (2.5 mM)

Table 1. Summary of the purification results of CGTase from *Thermoanaerobacter* sp.P4.

Purification step	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification fold
Supernatant	1000	168	172	1.02	100	1
Ammonium sulfate precipitate	15	2.04	44.5	21.8	25.8	21.37
Affinity chromatography	156	0.226	32.5	143.8	17.75	141

as donors and methyl α-D-glucopyranoside (10 mM) as acceptor (Alcalde et al., 1999). The incubation was carried out at 80°C for 10 min. Then, amyloglucosidase (from *Aspergillus niger*, Sigma) was used to convert the linear oligosaccharides formed during the reaction to glucose units at 37°C for 30 min. The amount of glucose formed was determined by DNS method (Forouchi and Gunn, 1983). One unit of enzyme activity was defined as the amount of enzyme converting 1 µmol of CD per minute under the assay conditions.

Protein determination

Protein content was determined according to Lowry et al. (1951) using Bovine serum albumin (Sigma, USA) as standard.

SDS-PAGE analysis

Molecular weight of CGTase was estimated by sodium dodecyl-sulphate polyacrylamide gel (SDS-PAGE) electrophoresis according to Laemmli (1970) on a vertical slab gel containing 10% polyacrylamide gel using Mini Protean electrophoresis apparatus (Biorad Laboratories). Standard molecular weight markers (Fermantas) ranging from 25 to 200 kDa were used for the esti-mation of molecular weight. The gel was stained with Coomassie Brilliant Blue R-20 (Merck, Germany). Molecular weight was calculated by using KODAK Molecular Imaging Software V.4.5.1 (Japan).

Optimum temperature and pH of the enzyme

Optimum pH for the activity of purified CGTase was determined by measuring the cyclization and hydrolyzing activities using soluble starch with designated buffers of sodium acetate buffer (50 mM, pH 4.0 to 6.0), tris HCl buffer (100 mM, pH 7.0 to 8.0) and glycine-NaOH buffer (100 mM, pH 9.0 to 11.0). Incubations were carried out at 80°C for 10 min by using 0.1 ml enzyme. Optimum temperature was determined by measuring the hydrolyzing and cyclization activities at temperatures between 40 to 95°C in the presence of soluble starch dissolved in 50 mM sodium acetate buffer at pH 6.0 and 0.1 ml of enzyme.

Thermal and pH stability of the enzyme

In order to determine pH stability of CGTase, the purified enzyme was incubated with varying buffers indicated above for 1 h at 80°C without substrate, and the remaining activity was determined using the cyclization method mentioned above. Temperature stability was determined by incubating purified enzyme in 50 mM sodium acetate buffer (pH 6.0) at 80, 90 and 100°C for 4 h without substrate, and residual cyclization activity was measured as mentioned in the enzyme assay section.

Effects of metals on the activity

Effects of metal ions on cyclization activity were investigated by incubating 0.1 ml purified enzyme with 0.2 ml of 50 mM sodium acetate buffer pH 6.0 containing 1 and 10 mM of $AlCl_3.6H_2O$ $AgNO_3$, $CoCl_3$, $CuSO_4.5H_2O$, $MgSO_4.7H_2O$, $MnCl.4H_2O$, $FeCl_2.4H_2O$, $BaCl_2.2H_2O$, KCl, $ZnSO_4.7H_2O$, and $HgSO_4$, $CaCl_2$. After the incubation for 30 min at 25°C, the residual enzyme activity for cyclization was measured.

Kinetic parameters

K_m and V_{max} values of the enzyme were determined by incubating 0.35 µg of purified CGTase in 1 ml 50 mM sodium acetate buffer at pH 6.0 containing various concentrations of soluble starch, ranging from 0.4 to 20 mg/ml at 80°C for 10 min. The K_m and V_{max} of the enzyme were determined from Lineweaver-Burk plot.

RESULTS

Purification of CGTase

The crude enzyme having a specific activity of 1.02 U/mg was purified in two steps including ammonium sulfate precipitation and affinity chromatography. Purification results of the enzyme are given in Table 1. After the purification with α-CD bound–epoxy activated Sepharose 6B affinity chromatography, the CGTase was purified 141 fold with a yield of 17.8%. The specific activity of the purified enzyme was found as 143.8 U/mg. Other than cyclization, hydrolysis and coupling activities of the enzyme were determined and they are given in Table 2 in comparison with some reported CGTases.

Single band was obtained on SDS-PAGE which confirms the purity of the enzyme. Molecular weight of the enzyme was estimated as 68.7 kDa on SDS-PAGE gel (Figure 1).

Properties of purified CGTase

Effect of pH and temperature on CGTase activity

Optimum pH and temperature of the enzyme was determined for both hydrolyzing and cyclization activities (Figures 2 and 3). Enzyme was active at a wide range of pH values having the maximum at pH 7.0 for both activities. Specific cyclization and hydrolyzing activities

Table 2. Specific enzymatic activities of CGTases from different sources

Strain	Cyclization (U/mg)	Hydrolyzing (U/mg)	Coupling (U/mg)		Reference
			α-CD	β-CD	
Thermoanaerobacter sp. P4*	157.3	55.3	399	238	This work
*T. thermosulfurigenes*EM1	86	23	-	74	(Wind et al., 1995)
Thermoanaerobacter sp.501	252	82	270	48	(Alcade et al., 1999)
Klebsiella pneumonia AS-22	523	0	498	-	(Gawande and Patkar 2001)
B. agaradhaerens LS-3C	14.8	7.75	7.37	3.9	(Martins and Hatti-Kaul, 2003)

Activities were determined at 80°C and pH 6.0 with soluble starch.

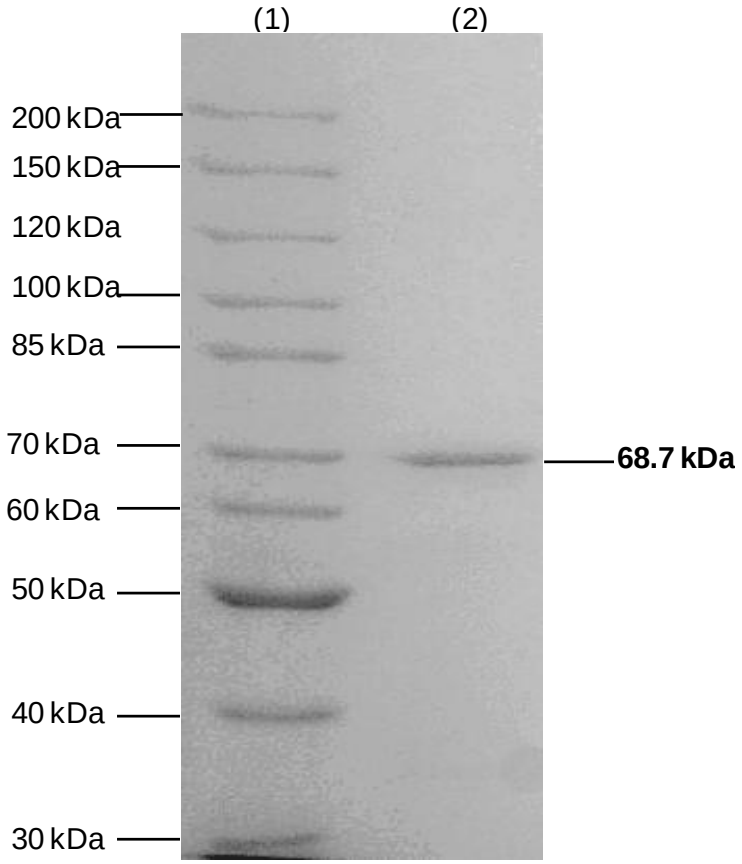

Figure 1. Determination of molecular weight of purified CGTase on SDS-PAGE. 1,Molecular weight standard; 2, purified CGTase.

were determined as 157.3 and 55.3 U/mg at this pH, respectively. It retained over 90% of its activities between pH 6.0 and pH 8.0. There was no complete inhibition at extreme pH values like pH 4.0 and pH 11.0 at which over 65 and 40% of both activities were retained, respectively (Figure 2).

The effect of temperature on the cyclization and hydrolyzing activities is illustrated in Figure 3. At temperatures below 70°C, both activities were quite low. The higher temperatures promoted both activities and maximum cyclization activity was obtained at 80 to 90°C and hydrolyzing activity at 90°C. The enzyme requires higher temperatures for hydrolyzing activity than cyclization which increased significantly with increasing temperatures and it was still high even at 95°C.

Effects of pH and temperature on CGTase stability

Figure 4 shows the stability of CGTase at varying pH values measured in terms of cyclization activity. Enzyme was stable within a broad pH range. It maintained over

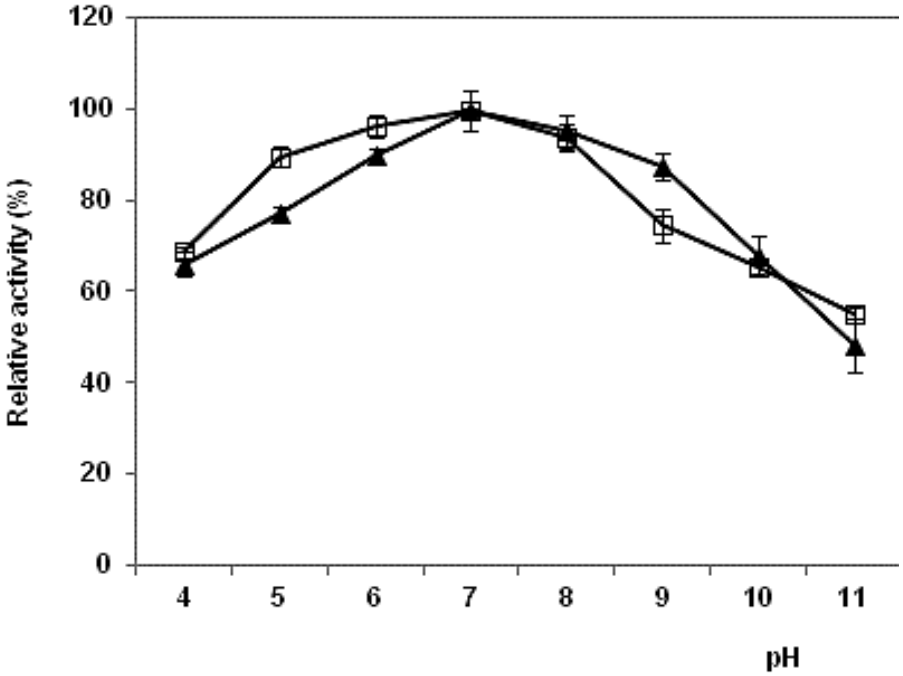

Figure 2. Effect of pH on the cyclization (▲) and hydrolyzing (□) activities of purified CGTase from *Thermoanaerobacter* sp. P4. 100% corresponds to 159.4 U/mg for cyclization activity and 58.1 U/mg for hydrolyzation activity. Incubations were carried out at 80°C with soluble starch.

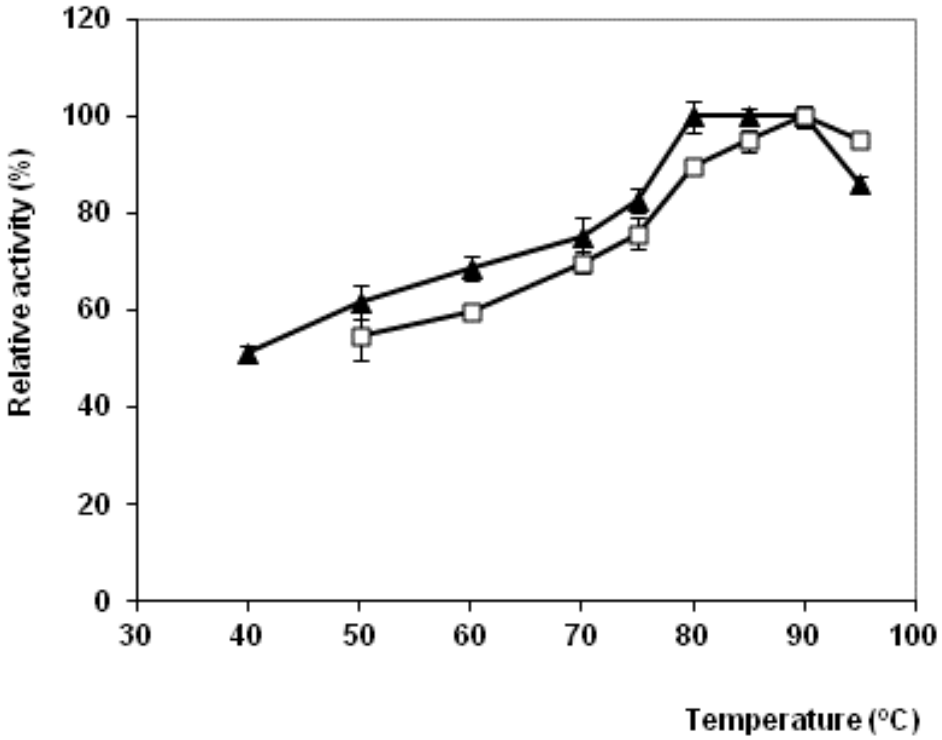

Figure 3. Effect of temperature on the cyclization (▲) and hydrolyzing (□) activities of purified CGTase from *Thermoanaerobacter* sp. P4. Activities were measured at pH 6.0 with soluble starch as substrate. 100% corresponds to 157.3 U/mg for cyclization activity and 55.3 U/mg for hydrolyzation activity.

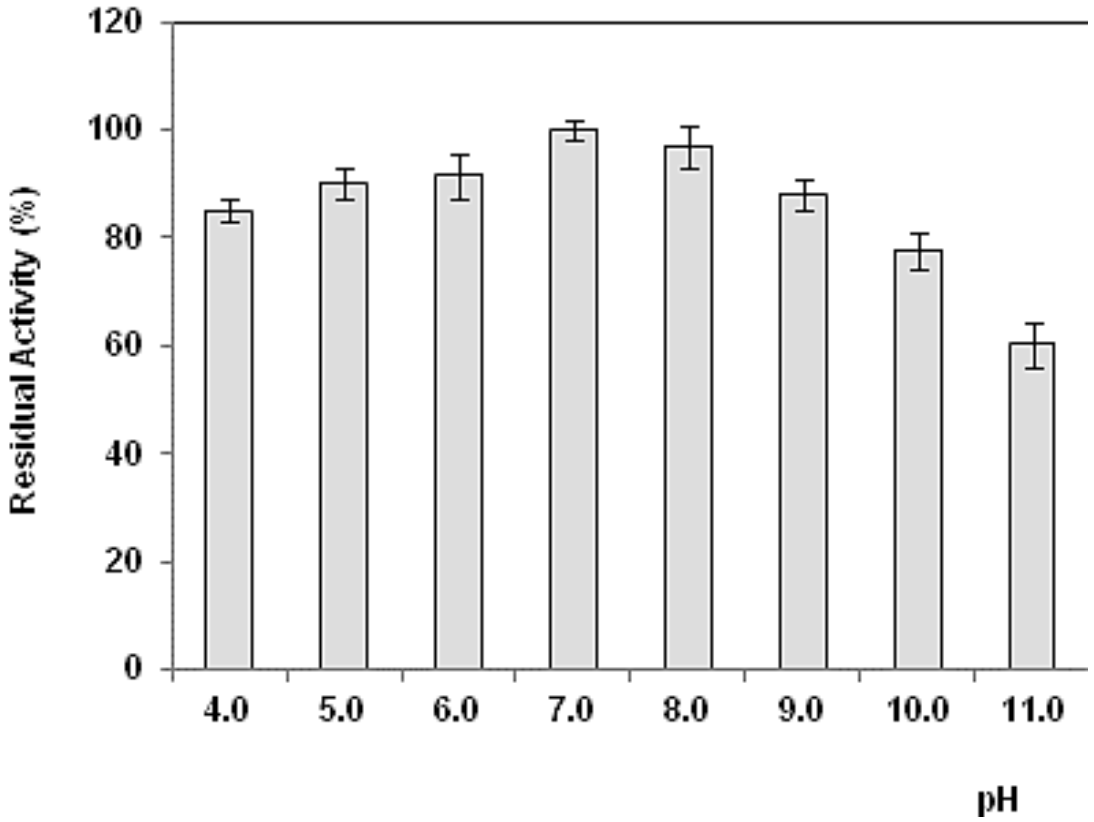

Figure 4. Effect of pH on the stability of purified CGTase from *Thermoanaerobacter* sp. P4 determined in terms of cyclization activity. The enzyme was incubated at 80°C in buffers having varying pH values for 1 h without substrate and cyclization assay was performed to determine residual activity. 100% corresponds to 157.3 U/mg specific activity.

80% of its activity at pH levels between 4.0 and 10.0. Lowest stability was observed at pH 11.0, at which 60% of the activity was retained.

Thermal stability of the enzyme was tested by incubating the enzyme at 80, 90 and 100°C for 4 h and residual activities were analyzed every 30 min during this period (Figure 5). Enzyme was fully stable at 80°C and no loss activity was observed at the end of 4 h of incubation. When the temperature was increased to 90°C, only 9% of loss in the activity was detected in 1 h. It lost 72% of its activity in 4 h at this temperature. The half life of the enzyme was 35 min at 100°C.

Effects of various metals on CGTase activity

Effects of some metals on CGTase activity were tested at 1 and 10 mM final concentrations of these metals, and the results are given in Table 3. CGTase was almost stable (over 84%) at 1 mM concentrations of the metals except for Hg and Ag, with which enzyme was inhibited 41 and 75%, respectively. As the concentration was increased to 10 mM, complete inhibition by $HgSO_4$ and $AgNO_3$ was detected.

Kinetic parameters of the enzyme

The kinetic parameters for the CGTase were determined using soluble starch at 80°C. The values of Km, Vmax and kcat were calculated as 1.96 ± 0.36 mg/ml, 161.3 ± 5.4 µmol/min/mg, and 184.7 ± 6.2 s^{-1}, respectively from Lineweaver-Burk plot (data not shown).

DISCUSSION

It is known that CGTases catalyze four different reactions named as cyclization, hydrolyzing, coupling and disproportionation. These activities vary depending on the source of organisms (Martins and Hatti-Kaul, 2003). In our study, cyclization, hydrolyzing and coupling activities of the purified enzyme were determined and it was demonstrated that the enzyme had all the activities measured (Table 2). Hence, it is a true CGTase. It was reported that CGTases from thermophilic anaerobic bacteria generally have higher hydrolysis activity compared with mesophilic strains such as *Bacillus* and *Klebsiella* (Alcalde et al., 1999; Gawande and Patkar, 2001). CGTase from *Thermoanaerobacter* sp P4 had

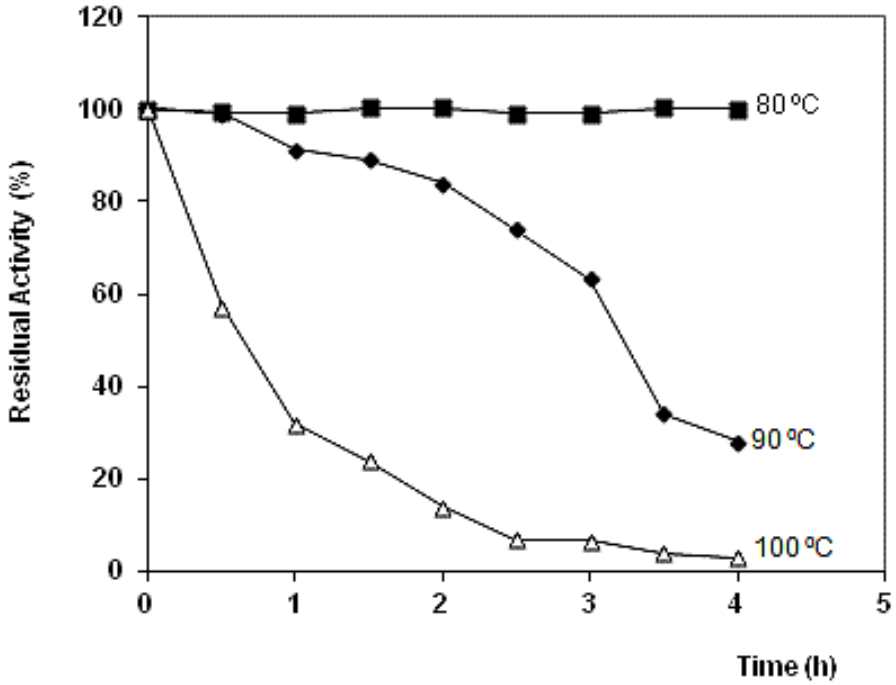

Figure 5. Effect of temperature on the stability of purified CGTase from *Thermoanaerobacter* sp. P4 determined in terms of cyclization activity. The enzyme was incubated at varying temperatures in sodium acetate buffer pH 6.0. 100 % corresponds to 157.3 U/mg specific activity

Table 3. Effects of 1 and 10 mM concentrations of various metals on the cyclization activity of *Thermoanaerobacter* sp. P4 CGTase.

Compound	Relative activity (%)	
	1 mM	10 mM
Control	100	100
$AlCl.6H_2O$	89	76
$AgNO_3$	75	0
$CoCl_3$	86	75
$CuSO_4.5H_2O$	90	67
$MgSO_4.7H_2O$	96	83
$MnCl.4H_2O$	96	67
$FeCl_2.4H_2O$	94	64
$BaCl_2.2H_2O$	96	78
KCl	95	75
$ZnSO_4.7H_2O$	98	75
$HgSO_4$	41	10
$CaCl_2$	107	102

Enzyme was incubated in 50 mM sodium buffer pH 6.0 in the presence of 1 and 10 mM of the metals at 25°C for 30 min and residual activities were measured.

55.3 U/mg which is as high as the other reported CGTases from thermophilic bacteria.

Molecular weight was estimated as 68.7 kDa on SDS-PAGE (Figure 1), which is in accord with the molecular weights of the reported CGTases. Even though there are some exceptions, most of the CGTases have molecular weights of around 70 kDa. Molecular weights of CGTases from *Thermoanaerobacterium thermosulfurigenes* EM1 (Wind et al., 1995), *Thermoanaerobacter* sp. 53627 (Norman and Jorgensen, 1992), alkalophilic *Anaerobranca gottschalkii* (Theimann et al., 2004), archeae *Thermococcus* sp B1001 (Tachibana et al., 1999), *Thermococcus kodakarensis* (Rashid et al., 2002) and *Pyrococcus furiosus* (Lee et al., 2007) were reported as 68, 75.5, 78, 83, 67 and 65 kDa, respectively.

The optimum pH and the temperature of the CGTase were determined in the supernatant of the bacterium and reported elsewhere (Avci and Dönmez, 2009). In this study, those from the purified CGTase have been also determined in order to confirm the properties of the enzyme before and after purification. Almost the same results were acquired in both studies. Purified CGTase displayed an optimum cyclization activity at 80 to 90°C which is similar to the optimum temperatures of thermophilic anaerobic *Thermoanaerobacterium thermosulfurigenes* EM1 (Wind et al., 1995) and *Thermoanaerobacter* sp. (Jorgensen et al., 1997). In general, extracellular enzymes may be stable at temperatures considerably higher than the growth temperatures (Cowan and Lafuente, 2011). Activity at 95°C was also high enough as an indication of the

resistance of the enzyme to the high temperature applications. When temperature was raised from 90 to 95°C, thermal inactivation was only 13% (Figure 3). Enzyme exhibited hydrolyzing activity at higher temperatures than that of the cyclization activity, which is maximal at 90 to 95°C. It seems that hydrolyzing activities at higher temperatures than cyclization activities is a common property of CGTases. Similarly, CGTase from *T. thermosulfurigenes* EM1 had same characteristic (Wind et al., 1995).

CGTase from *Thermoanaerobacter* sp. P4 is highly thermostable possessing no activity loss at 80°C during 24 h incubation period (data not shown). The thermal stabilities of the CGTases from other thermophilic anaerobic bacteria were reported by some researchers. Norman and Jorgensen (1992) found that CGTase from *Thermoanaerobacter* sp ATCC 53627 retained 95 to 100% of its activity at 75°C for 60 min. Wind et al. (1995) tested the thermal stability of *T. thermosulfurigenes* EM1 in the presence of Paselli SA2, and found 10% loss at 80°C and 35% at 90°C after 5 h. It must be pointed out that thermal stability was measured without addition of any substrate into the medium in this study. It was stated that substrate addition enhances the stability of CGTase (Martins and Hatti-Kaul, 2003; Theimann et al., 2004; Wind et al., 1995). Unlike our enzyme that can retain 63 and 35% of its activity at 90°C without any supplementation like starch and calcium, CGTase from *T. thermosulfurigenes* EM1 has been reported to lose completely its activity in 1 h at the same conditions (Wind et al., 1995). CGTase from *Thermoanaerobacter* sp. P4 possessed similar thermal characteristics to other thermostable CGTases except CGTase from hyper-thermophilic archaeon *Thermococcus* sp. that has the highest CGTase activity (100°C) among all the reported CGTases (Tachibana et al., 1999).

The inhibition of the CGTase activity by most of the metals except $CaCl_2$, which increases the activity and stability, is known. The inhibitory effect is attributed to the metal catalyzed oxidation of amino acid residues essential for the enzyme activity (Jemli et al., 2007; Martins and Hatti-Kaul, 2003; Sian et al., 2005). CGTase activity of the enzyme was also enhanced by the addition of 1 mM and 10 mM concentrations of $CaCl_2$ which led to 7 and 2% increases in the activity, respectively. Effect of $CaCl_2$ on the activity of CGTase was studied by various researchers (Rashid et al., 2002; Theimann et al., 2004; Wind et al., 1995) and it has been reported that stimulation of CGTase activity in the presence of $CaCl_2$ is a common phenomenon among CGTases (Martins and Hatti-Kaul, 2003).

The CGTases from various sources exhibit different Km values for soluble starch ranging from 0.52 to 21.2 mg/ml (Gawande and Patkar, 2001; Martins and Hatti-Kaul, 2002; Rahman et al., 2006; Stavn and Granum, 1979). Km value of CGTase from *Thermoanaerobacter* sp. P4 was calculated as 1.95 mg/ml indicating that the enzyme has high affinity for soluble starch.

Conclusion

A new CGTase from *Thermoanaerobacter* sp. P4 was purified and characterized in terms of molecular weight, pH, temperature, effect of metals and kinetic parameters. The enzyme shows high activities at 80 to 95°C which is desirable in the industrial productions of CDs. Another promising property of the enzyme is the higher hydrolyzing activities at elevated temperatures. Thus, considering the limited amounts of thermostable CGTases, this enzyme can be a potential for industrial application.

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