J. Microbiol. Biotechnol. (2002), **12**(6), 921–928

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Cloning and Characterization of Cycloinulooligosaccharide Fructanotransferase (CFTase) from *Bacillus polymyxa* MGL21

JEON, SUNG-JONG[1], DONG-JU YOU[3], HYUN-JU KWON[2], SHIGENORI KANAYA[2], NAMIO KUNIHIRO[2], KWANG-HYEON KIM[3], YOUNG-HEE KIM[3], AND BYUNG-WOO KIM[3]*

[1]*The special division for Human Life Technology, National Institute of Advanced Industrial Science and Technology (Kansai), Osaka 563-8577, Japan*
[2]*Department of Material and Life Science, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 567-0871, Japan*
[3]*Department of Microbiology, Graduate School, Dongeui University, 614-714, Korea*

Received: August 19, 2002
Accepted: November 15, 2002

Abstract Microorganism producing extracellular CFTase was isolated from soil and designated as *Bacillus polymyxa* MGL21. The gene encoding the CFTase (*cft*) from *B. polymyxa* MGL21 was cloned and sequenced. The ORF of the *cft* gene was composed of 3,999 nucleotides, encoding a protein (1,333 amino acids) with a predicted molecular mass of 149,375 Da. Sequence analysis indicated that CFTase was divided into five distinct regions. CFTase contained three regions of repeat sequences at the N-terminus and C-terminus. The endo-inulinase region of homology (ERH) of CFTase was similar to that of *Pseudomonas mucidolens* endo-inulinase (50% identity, 259 amino acids). Furthermore, CFTase possessed a highly conserved core region, which is considered to be functional for the hydrolysis reaction of inulin. The *cft* gene was expressed in a His-tagged form in *Escherichia coli* cells, and the His-tagged CFTase was purified to homogeneity. The optimal temperature and pH for CFTase activity were found to be 50°C and 9.0, respectively. The enzyme activity was completely inhibited by 10 mM Ag^{+} and Cu^{2+}. Thin-layer chromatography analyses indicated that CFTase catalyzed not only the cyclization reaction but also disproportionation and hydrolysis reactions as well.

Key words: Cyclofructan, cycloinulooligosaccharide fructanotransferase, CFTase, transfructosylation, inulin

Cycloinulooligosaccharides (cyclofructans) are the cyclic oligosaccharides which consist of six to eight molecules of β-(2→1)-linked D-fructofuranoses [cycloinulohexaose (CF6), cycloinuloheptaose (CF7), and cycloinulooctaose (CF8)]. Cyclofructans have a characteristic crown ether in the central part of the molecule that can bind cationic molecules via charge-dipole electrostatic interactions [18]. Cyclofructans also have stabilizing effects on various materials during the freezing and thawing process [3, 20]. Accordingly, cyclofructans are expected to be utilized extensively in the medical, food, and chemical fields.

Cyclofructans are synthesized from inulin by cycloinulooligosaccharide fructanotransferase (CFTase). Inulin is a polyfructan consisting of a linear β-(2→1)-linked polyfructose chain with a terminal glucose residue. It is found as a carbohydrate reservoir in various plants such as chicory, dahlia, and Jerusalem artichoke. CFTase catalyzes degradation of inulin into cycloinulooligosaccharides by intramolecular transfructosylation, and also catalyzes intermolecular transfructosylation between β-(2→1)-fructooligosaccharides by disproportionation and coupling reactions [4, 6]. Currently, three microorganisms, *Bacillus circulans* OKUMZ 31B [6], *B. circulans* MCI-2554 [12], and *B. macerans* CFC1 [10], have been found to produce CFTase.

In this study, we isolated a novel microorganism that produced an extracellular CFTase from soil. This bacterial strain, designated as MGL21, was identified as *B. polymyxa*. In addition, we cloned the gene encoding a CFTase from the isolated strain and characterized the recombinant protein.

MATERIALS AND METHODS

Bacterial Strains

The microorganism producing CFTase was isolated from soil cultivating Jerusalem artichoke. The soil samples were diluted with sterilized water, and incubated at 30°C for 2

*Corresponding author
Phone: 82-51-890-1536; Fax: 82-51-891-7740;
E-mail: bwkim@dongeui.ac.kr

days in the Luria-Bertani (LB) medium containing 1% (w/v) inulin. The incubated samples were inoculated to plates of the LB agar. The production of CFTase was detected by TTC staining as described below. After incubation at 37°C for 2 days, 10 colonies which formed a red zone were isolated, inoculated into a medium that contained 0.2 g of inulin, 0.05 g of yeast extract, 0.05 g of $(NH_4)_2HPO_4$, 0.02 g of $NH_4H_2PO_4$, 0.005 g of KCl, 0.005 g of $MnCl_2 \cdot 4H_2O$, 0.005 g of $MgSO_4 \cdot 7H_2O$, and 0.005 g of $FeSO_4 \cdot 7H_2O$ in tap water (pH 7.0), and incubated at 37°C for 2 days. The cells were centrifuged and the supernatant was used as the crude enzyme source. The formation of cyclofructan was detected by using thin-layer chromatography (TLC) and also by using HPLC as described below. The microorganism which produced high levels of cyclofructan was selected and identified on the basis of *Bergey's Manual of Systematic Bacteriology* [17] and GP MicroPlate™ (Biolog, Hayward, CA, U.S.A.).

Escherichia coli DH5α [F^-, ϕ80d*lacZ*ΔM15, Δ(*lacZYA-argF*) U169, *deoR*, *recA*1, *endA*1, *hsdR*17 ($r_k^- m_k^+$), *PhoA*, *supE*44, *thi*-1, *gyrA*96, *relA*1] and *E. coli* HMS174(DE3) pLysS [F^-, *recA*1, *hsdR* ($r_{k12}^- m_{k12}^+$), *Rif*^{R} (DE3) pLysS] were used as the host strains for gene cloning and gene expression, respectively.

16S rRNA Gene Cloning and Sequencing

The 16S rRNA gene was amplified by PCR in 30 cycles with a GeneAmp PCR System 2400 (Perkin Elmer, Foster City, CA, U.S.A.) by using AmpliTaq DNA polymerase (Takara Shuzo, Kyoto, Japan) as described previously [7, 13]. The PCR amplification primers used were 16S-7 (5'-AAGAGTTTGATCATGGC-3') and 16S-1510 (5'-AGGAGGTGATCCAACCGCAG-3'), which represent a 16S rRNA gene of *E. coli* (EMBL/GenBank/DDBJ accession number X80721). Chromosomal DNA used as a template was prepared by the sarkosyl method and purified by CsCl equilibrium density gradient ultracentrifugation [1].

Purification of Cyclofructan

The crude enzyme suspension was incubated with 6% (w/v) inulin in 50 mM phosphate buffer (pH 7.5) at 45°C for 3 h. The reaction was stopped by boiling for 5 min, and the reaction mixture was concentrated under reduced pressure. The concentrated sample was purified by gel filtration by using Sephadex G-15 (Amersham Biosciences, Piscataway, NJ, U.S.A.). The fractions containing inulooligosaccharides were detected by the Somogyi-Nelson method [14], and cyclofructans were detected by HPLC [8, 11]. Conditions for HPLC analysis were as follows: column, TSK-GEL AMIDE-80 (4.6×250, TOSOH, Tokyo, Japan); detector, RI detector (Model 410, Waters, Milford, MA, U.S.A.); mobile phase, acetonitrile:water (65:35); flow rate, 1 ml/min; and column temperature, 65°C.

Cloning and DNA Sequencing

DNA fragment containing *cft* gene was obtained by the shot-gun cloning method. *Eco*RI-digested chromosomal DNA of *B. polymyxa* MGL21 was ligated with previously *Eco*RI-cleaved pUC18 (Takara) by using standard recombinant DNA techniques [15]. The ligation mixture was transformed into *E. coli* DH5α and plated on the LB medium containing ampicillin, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and isopropyl-β-D-thiogalactopyranoside (IPTG). After 16–24 h of incubation at 37°C, white colonies were isolated and screened by TLC analysis. The *cft* gene was cloned as a 11.5 kb *Eco*RI fragment in the pUC18, and the resulting recombinant plasmid was designated as pDI 1.

DNA sequencing analysis was performed by applying the dideoxy chain-termination method [16] with an ABI PRISM 310 genetic analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA, U.S.A.). The nucleotide and deduced amino acid sequences were analyzed by a DNASIS software (Hitachi Softwares Engineering, Yokohama, Japan).

Protein Expression and Purification

For *cft* gene expression, DNA fragment containing the *cft* gene was amplified by PCR by using the chromosomal DNA as template and two primers (primer 1, 5'-AGGAAAGCCAAAGGAAGAAAATGTTGG-3'; primer 2, 5'-AACTCGAGGTTGTTGGTTTTCTTC-3'). DNA amplification was performed by using KOD1 DNA polymerase (Toyobo, Osaka, Japan) with the following program: (i) 10 cycles of 98°C for 60 s, 55°C for 30 s, and 74°C for 60 s; (ii) 20 cycles of 98°C for 15 s, 55°C for 2 s, and 72°C for 30 s. The expression vector pET-28a was digested with *Nco*I, treated with T4 DNA polymerase to fill in the cohesive ends, and again digested with *Xho*I. An amplified 4.0-kb DNA fragment was digested with restriction enzymes *Xho*I (the *Xho*I site in primer 2 is underlined) and inserted into the pET-28a that contained a region coding for the His-tag sequence (Novagen, Madison, WI, U.S.A.). The resulting plasmid was designated as pET-CFT.

His-tagged CFTase was expressed by growing *E. coli* harboring pET-CFT to approximately 0.4 turbidity at 600 nm and then inducing expression with 0.5 mM IPTG for 4 h at 37°C. The cells were then disrupted by sonication and the supernatant was recovered by centrifugation at 30,000 ×g for 30 min at 4°C. The supernatant was applied to a metal chelating column (Amersham Biosciences) and washed with at least 10 column volumes of the resuspension buffer (20 mM Tris-HCl, pH 7.5, and 0.5 M NaOH), and the fusion protein was finally eluted with a linear gradient of 0 to 300 mM imidazole in a resuspension buffer at a flow rate of 1 ml/min by using the FPLC system (Amersham Biosciences). The purity of fusion protein was assessed on SDS-PAGE.

Enzyme Assay

To isolate a bacterial strain capable of degrading inulin, TTC staining was carried out by spraying the agar plate with TTC reagent (0.1% triphenyl tetrazolium chloride in 0.5 M sodium hydroxide) for 20 min in the dark. After washing in 0.1 M acetate buffer (pH 5.0), the extracellular production of inulinase was confirmed by the appearance of red zone that was formed around the colonies on the LB agar supplemented with inulin. CFTase activity was measured by incubating the enzyme with 2% inulin in 50 mM phosphate buffer (pH 7.5) at 45°C for 1 h. The reaction was stopped by boiling for 5 min, and the reaction products were analyzed for inulin by TLC. Aliquots (5 μl) of the reaction mixtures were chromatographed on a silica gel plate (Merck Co., Berlin, Germany) three times with *n*-butanol-isopropanol-water (3:12:4, by vol) [2. 19], and the products were detected by spraying the plate with urea reagent (93.22 ml of *n*-BuOH, 6.78 ml of phosphoric acid, 5 ml of EtOH, and 3 g urea) and treating it at 110°C for 10 min.

Nucleotide Sequence Accession Number

The nucleotide sequence of the *cft* gene from *B. polymyxa* MGL21 has been submitted to the DDBJ/EMBL/GenBank nucleotide sequence database under the accession number of AY077612.

RESULTS AND DISCUSSION

Isolation and Identification of Microorganism

The microorganism producing CFTase was isolated from soil by using a culture containing inulin as the sole carbon source. In the screening stage, we selected a microorganism which produced inulooligosaccharides from inulin by TLC analysis, and designated it as the strain MGL21. The products obtained from the crude enzyme preparation with inulin were purified by gel filtration and analyzed by HPLC. The crude enzyme preparation produced CF6, CF7, and CF8 in a ratio of 72:27:1 (Fig. 1). The isolated strain MGL21 was recognized as a Gram-positive and motility-positive bacterium (Table 1). As shown in Table 1, the microorganism was identified as a strain of *Bacillus* sp. from the physiological and biochemical characteristics.

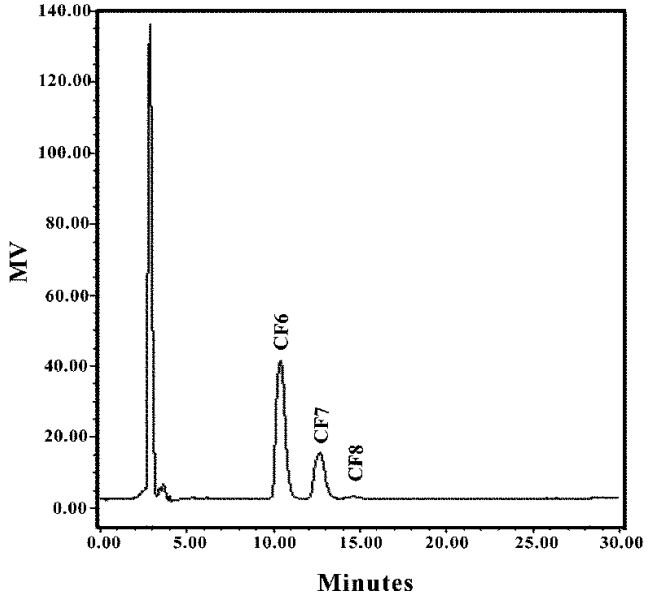

Fig. 1. HPLC chromatogram of the CF6, CF7, and CF8 in the enzyme reaction mixture.

Table 1. Physiological and biochemical properties of the isolated strain MGL21.

Characteristics	Strains MGL21
Gram staining	+
Motility	+
Catalase	+
Anaerobic growth	+
Voges-Proskauer test	+
Hydrolysis of :	
Gelatin	+
Esculin	+
Starch	+
Formation of	
Indole	–
Dihydroxyacetone	+
Growth on nutrient broth at pH 5.7	+
Maximum growth temperature, °C	20–45°C
Maximum NaCl tolerance, %	0–1%
Carbon utilization	
D-fructose	+
D-glucose	+
Lactulose	+
D-psicose	+
Methyl pyruvate	+

Sequencing of the 16S rRNA Gene

A 1.5 kbp DNA fragment from the strain carrying more than 95% of the 16S rRNA gene was cloned and its partial nucleotide sequence was determined (500 bp from both sides of this DNA fragment). The nucleotide sequences of the 5'-terminal and 3'-terminal 500 bp DNA fragments are deposited in DDBJ/EMBL/GenBank with accession numbers of AY135393 and AY135394, respectively. The nucleotide sequence of the 16S rRNA gene of this strain showed the highest identity of 98.0% to that of *B. polymyxa*. This high score affiliated the strain MGL21 with the order *Bacillales*, family *Bacillaceae*.

Cloning and Sequence Analysis of the *B. polymyxa* CFTase Gene

In order to obtain the *cft* gene from *B. polymyxa* MGL21, the shot-gun method was used for the cloning experiment as described in Materials and Methods. A plasmid harboring a 11.5 kb *Eco*RI fragment was obtained and the

nucleotide sequence of the entire open reading frame (ORF) was determined. The DNA and the deduced amino acid sequences are shown in Fig. 2. The ORF is preceded by a putative ribosomal binding site (5'-AGGAGG-3', 8 nucleotides upstream of the initiation codon ATG). The ORF encodes a protein of 1,333 amino acids with a

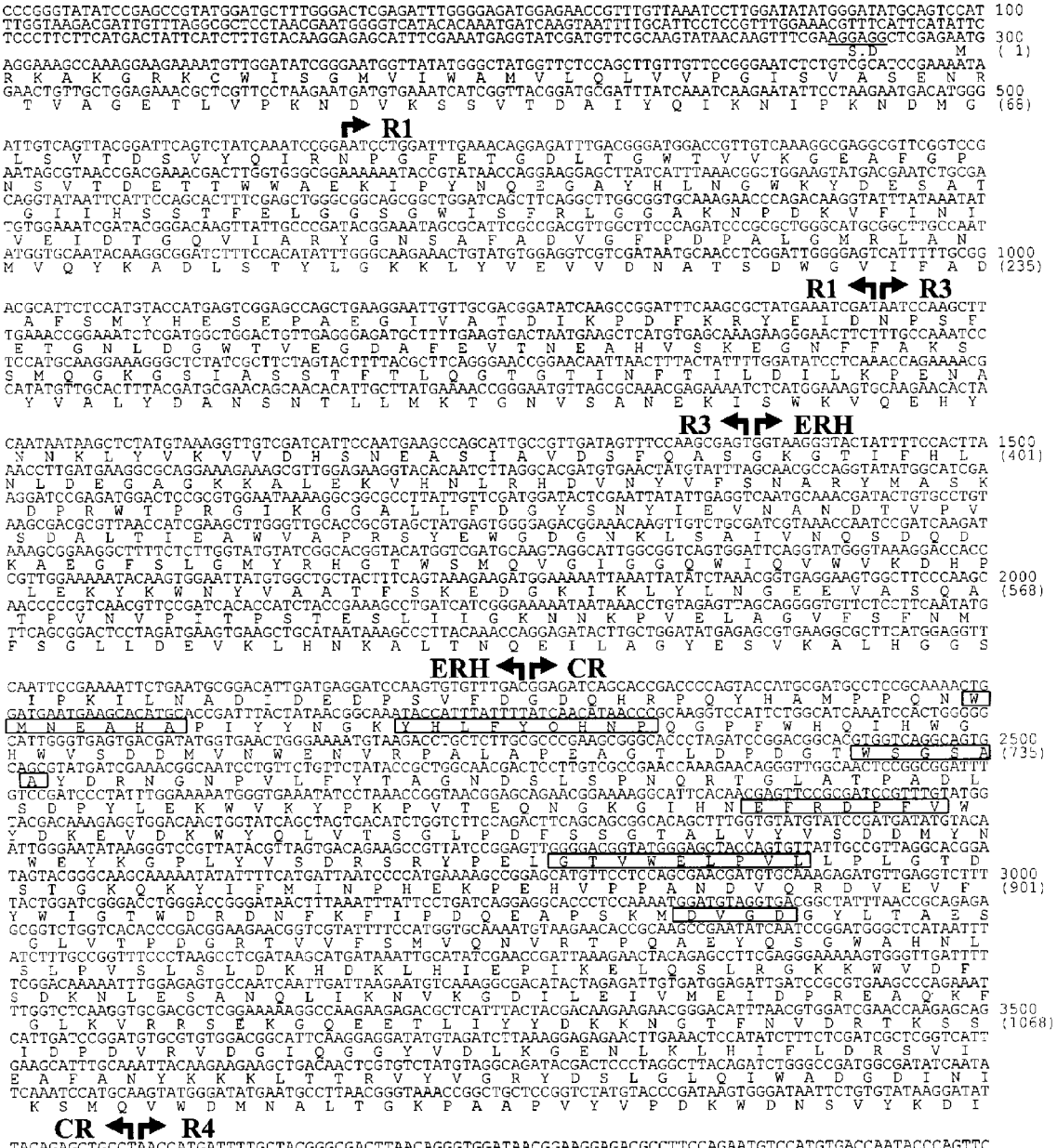

Fig. 2. Nucleotide and deduced amino acid sequences of the *B. polymyxa cft* gene.
A putative Shine-Dalgarno (SD) sequence is underlined. Six highly conserved regions are boxed. R1, R3 and R4, regions of repeat sequences; ERH, endo-inulinase region of homology; CR, core region.

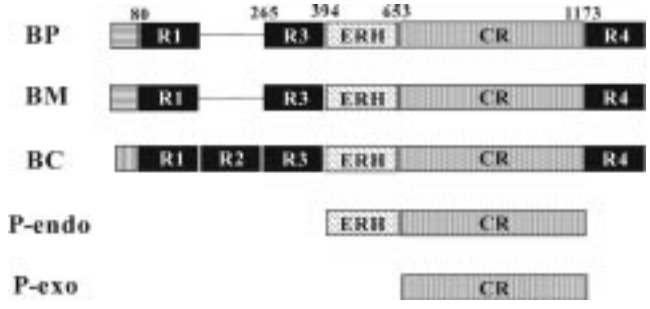

Fig. 3. Schematic drawings of the alignment of *B. polymyxa* CFTase with its homologues.
R1 to R4, regions of repeat sequences; ERH, endo-inulinase region of homology; CR, core region; BP, *B. polymyxa* CFTase; BM, *B. macerans* CFC1 CFTase; BC, *B. circulans* MCI-2554 CFTase; P-endo, *P. mucidolens* endo-inulinase; P-exo, *P. mucidolens* exo-inulinase.

predicted molecular mass of 149,375 Da and an estimated isoelectric point of 5.16. The ORF has been designated as the *cft* gene. Six highly conserved regions which had been reported in the inulin, levan, and sucrose hydrolyzing enzymes were also observed in CFTase from *B. polymyxa* (Fig. 2).

Figure 3 shows schematic drawings of the alignment of *B. polymyxa* CFTase with its homologues. The deduced amino acid sequence of CFTase from *B. polymyxa* was shown to be the most homologous to that (96% identity and 98% similarity) from *B. macerans* CFC1 and showed a relatively high homology to CFTase (73% identity and 84% similarity) of *B. circulans* MCI-2554. Also, it was found to be homologous to members of the β-fructofuranosidase family (*P. mucidolens* endo-inulinase, exo-inulinase, *Kluyveromyces marxianus* inulinase, and *B. subtilis* levanase). Sequence analysis indicated that CFTase from *B. polymyxa* was divided into five distinct regions (Fig. 3). CFTase and inulinase were found to possess a highly conserved core region (CR). This region is considered to be functional for hydrolysis reaction of inulin, since the core region has a sequence similar to that of β-fructofuranosidase. Endo-inulinase region of homology (ERH) was only found in CFTase as well as endo-inulinase, and ERH of *B. polymyxa* CFTase was similar to that of *P. mucidolens* endo-inulinase (50% identity, 259 amino acids). Hence, ERH is considered to be involved in endo-inulinase activity. The most striking difference between CFTase and inulinase was found in the N-terminal and C-terminal regions. CFTases have three or four regions of repeat sequences, whereas inulinases do not contain these regions. Therefore, it suggests that the cyclization reaction was conferred by the region of repeat sequences. Furthermore, it has been described that the N-terminal 358-residue domain of CFT150 from *B. macerans* might actually play a role in the substrate binding of the enzyme [10]. This domain is highly homologous (93% identity) to two regions of repeated sequences at the N-terminus of *B. polymyxa* CFTase. In order to understand the functions of the N- and C-terminal regions of the CFTase, we constructed three deletion mutants in which DNA regions encoding a part of the repeat sequences of the enzyme were deleted, and we are presently in the process of analyzing the characteristics of the mutant enzymes.

Overexpression and Purification of the Recombinant CFTase

B. polymyxa CFTase was expressed in a His-tagged form in the pET-28a, as described in Materials and Methods. When the cells harboring pET-CFT was induced only by IPTG, a significant CFTase activity was detected. The cells were harvested and disrupted by sonication, and the protein was purified by a single step on a nickel-affinity column [21]. SDS-PAGE analysis of the purified protein resulted in a single band with a mobility corresponding to a molecular mass of approximately 58 kDa (Fig. 4), in good agreement with the size calculated from the amino acid sequence for His-tagged CFTase (57.7 kDa). Purified enzyme was used for further enzymatic characterization.

Characterization of Purified CFTase

The enzyme activity was measured in the pH range of 5.0 to 10.0. As shown in Fig. 5, maximal activity was observed at pH 9.0. When the enzyme was incubated at pH 7–10 for 3 h, more than 90% of the CFTase activity remained. The effect of temperature on the enzyme activity

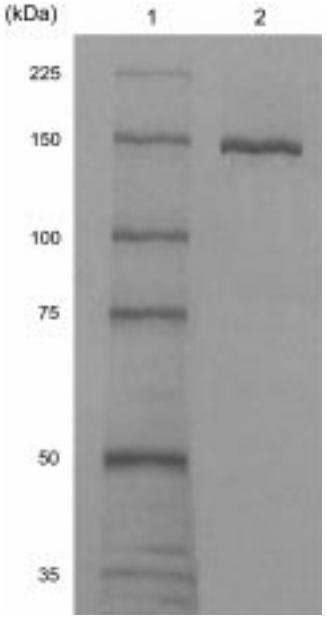

Fig. 4. Analysis of the purified fusion CFTase on 0.1% SDS-10% PAGE.
Lanes 1, Molecular mass marker consists of 9 precisely sized recombinant protein of 10,000, 15,000, 25,000, 35,000, 50,000, 75,000, 100,000, 150,000, and 225,000 (Novagen, Madison, WI, U.S.A.); lanes 2, His-tagged CFTase.

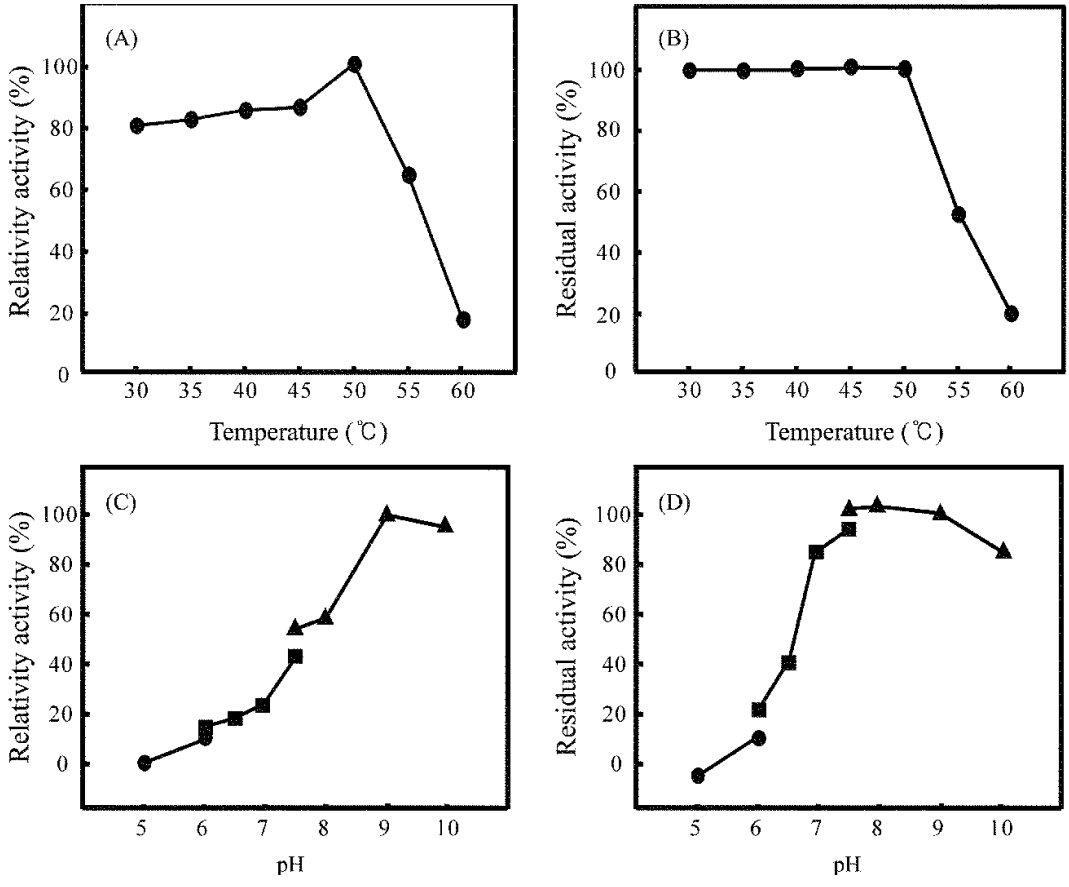

Fig. 5. Effects of temperature and pH on recombinant His-tagged CFTase activity.
The CFTase activity was measured at different temperatures (A). For thermostability test, an enzyme suspension was incubated at each temperature for 3 h, and then assayed for activity at 45°C (B). The CFTase activity was measured at different pHs (C). The enzyme was preincubated with various pH buffers for 3 h at 4°C and the residual activity was measured (D) (●, 50 mM acetate buffer; ■, 50 mM phosphate buffer; ▲, 50 mM Tris buffer).

was observed in the range of 30°C to 60°C. The enzyme showed maximal activity at 50°C, and it was stable up to 50°C for 3 h, while only 50% of the activity remained at 55°C for 3 h.

The optimal temperature and pH of the CFTase from *B. polymyxa* were different from those of CFTases from *B. macerans* CFC1 (45°C, pH 7.5) and *B. circulans* OKUMZ31B (40°C, pH 7.5) [8, 5]. The CFTase activity was completely inhibited in the presence of 10 mM Cu^{2+} or Ag^{+}, whereas Mg^{2+}, Mn^{2+}, and Fe^{2+} had no significant influence on the CFTase activity (data not shown). In order to understand the functional properties of *B. polymyxa* CFTase, we analyzed the reaction products with substrates such as inulin, 1-β-fructofuranosyl nystose (GF4) and cycloinulohexaose (CF6), and the reaction products were analyzed by TLC (Fig. 6). When inulin was used as a substrate, cyclofructan (CF6) was the major product by the cyclization reaction of the enzyme (Fig. 6A). When GF4 was used as a substrate, the first product was GF7, however, CF6, inulotriose (F3), and sucrose (GF) were also produced with comparable efficiencies (Fig. 6B). The formation of GF7 from GF4 was considered to be caused by a disproportionation reaction through an intermolecular transfructosylation. When CF6 was used as a substrate, F3 and inulotetraose (F4) were detected after 18 h of the reaction (Fig. 6C). These results indicate that CFTase can catalyze the decomposition of CF6. As described above, CFTase catalyzes not only the cyclization reaction but also disproportionation and hydrolysis reactions as well. The functional properties of *B. polymyxa* CFTase are similar to those of other CFTases from *B. circulans* OKUMZ31B and *B. macerans* CFC1 [4, 8, 9]. Thus, it can be deduced that *B. polymyxa* CFTase and the two other CFTases have similar structures and catalytic mechanisms. In view of industrial utilization, CFTase may also be used for the production of various sugar derivatives and synthesis of functional oligosaccharides.

In this study, we confirmed the functional properties of CFTase from *B. polymyxa*, and compared the amino acid sequence of CFTase with inulinases. Further analysis to

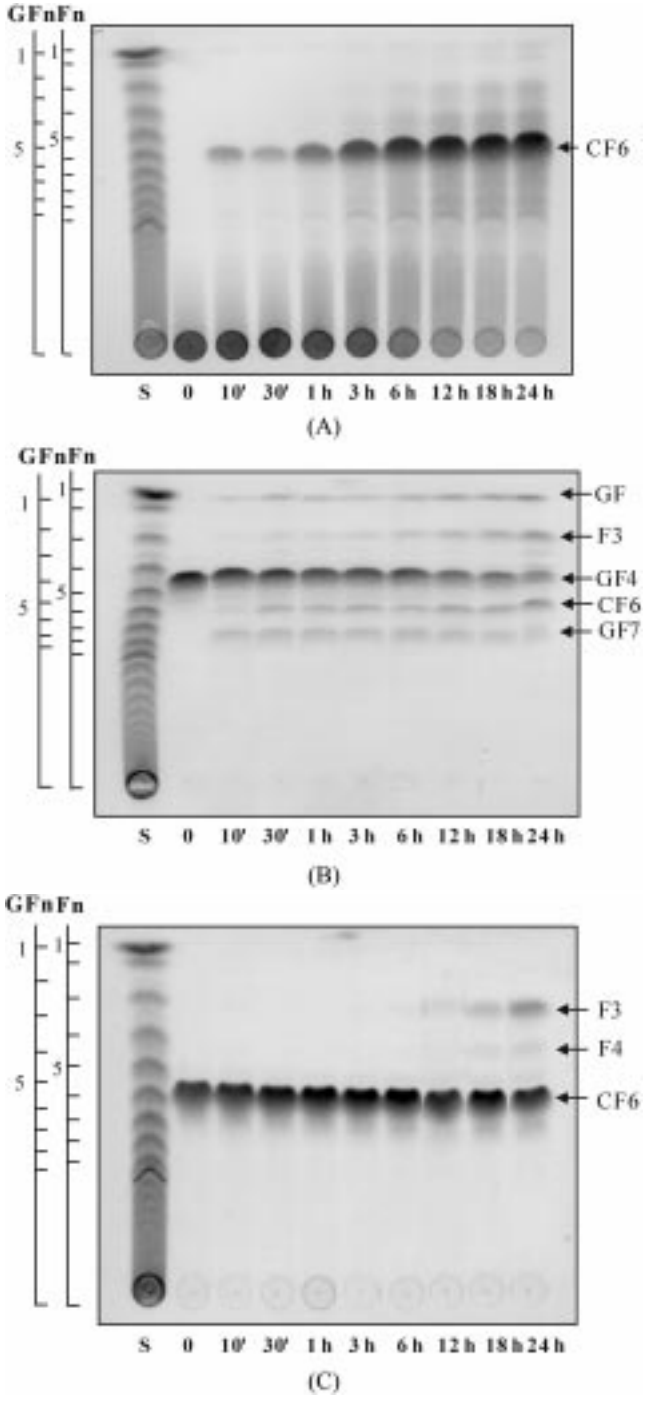

Fig. 6. TLC analysis of the reaction products by *B. polymyxa* CFTase with various substrates.
The reaction mixture containing 2% inulin (A), 0.5% GF4 (B), or 0.5% CF6 (C) in 50 mM phosphate buffer (pH 7.5) was incubated with the enzyme at 45°C. The reaction products at 0, 0.17, 0.5, 1, 3, 6, 12, 18, 24 h of incubation were analyzed. Positions of inulooligosaccharides (Fx) and glucofructooligosaccharides (GFx) are indicated on the ordinate. Lanes S, partial acid-hydrolysate of inulin.

understand the biochemical characteristics of this enzyme is in progress.

Acknowledgment

This work was supported by the Dongeui University research grant, 2000.

REFERENCES

1. Imanaka, T., T. Tanaka, H. Tsunekawa, and S. Aiba. 1981. Cloning of the genes for penicillinase, *penP* and *penI*, of *Bacillus licheniformis* in some vector plasmids and their expression in *Escherichia coli*, *Bacillus subtilis* and *Bacillus licheniformis*. *J. Bacteriol*. **147:** 776–786.
2. Jeong, T. H., H. O. Kim, J. N. Park, H. J. Lee, D. J. Shin, H. B. Lee, S. B. Chun, and S. Bai. 2001. Cloning and sequencing of the β-amylase gene from *Paenibacillus* sp. and its expression in *Saccharomyces cerevisiae*. *J. Microbiol. Biotechnol*. **11:** 65–71.
3. Kanai, T., N. Ueki, T. Kawaguchi, Y. Teranishi, H. Atomi, C. Tomorbaatar, M. Ueda, and A. Tanaka. 1997. Recombinant thermostable cycloinulo-oligosaccharide fructanotransferase produced by *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol*. **63:** 4956–4960.
4. Kawamura, M. and T. Uchiyama. 1993. Reaction catalyzed by cycloinulo-oligosaccharide fructanotransferase. *Biosci. Biotechnol. Biochem*. **57:** 343.
5. Kawamura, M. and T. Uchiyama. 1994. Purification and some properties of cycloinulo-oligosaccharide fructanotransferase from *Bacillus circulans* OKUMZ 31B. *Carbohydr. Res*. **260:** 297–304.
6. Kawamura, M., T. Uchiyama, T. Kuramoto, Y. Tamura, and K. Mizutani. 1989. Formation of a cycloinulo-oligosaccharide from inulin by an extracellular enzyme of *Bacillus circulans* OKUMZ 31B. *Carbohydr. Res*. **192:** 83–90.
7. Kim, D. J., M. Morikawa, M. Takagi, and T. Imanaka. 1995. Gene cloning and characterization of thermostable peptidyl prolyl cis-trans isomerase (PPIase) from *Bacillus stearothermophilus*. *J. Ferment. Bioeng*. **79:** 87–94.
8. Kim, H. Y. and Y. J. Choi. 1998. Purification and characterization of cycloinulooligosaccharide fructanotransferase from *Bacillus macerans* CFC1. *J. Microbiol. Biotechnol*. **8:** 251–257.
9. Kim, H. Y. and Y. J. Choi. 2000. Regulation of cycloinulooligosaccharide fructanotransferase synthesis in *Bacillus macerans* and *Bacillus subtilis*. *J. Microbiol. Biotechnol*. **10:** 877–880.
10. Kim, H. Y. and Y. J. Choi. 2001. Molecular characterization of cycloinulooligosaccharide fructanotransferase from *Bacillus macerans*. *Appl. Environ. Microbiol*. **67:** 995–1000.
11. Kim, H. Y., J. B. Park, Y. M. Kwon, and Y. J. Choi. 1996. Production of cycloinulooligosaccharide fructanotransferase (CFTase) from *Bacillus* sp. CFC1. *J. Microbiol. Biotechnol*. **6:** 397–401.
12. Kushibe, S., R. Sashida, and Y. Morimoto. 1994. Production of cyclofructan from inulin by *Bacillus circulans* MCI-2554. *Biosci. Biotechnol. Biochem*. **58:** 1136–1138.
13. Lee, W. J. and K. S. Bae. 2001. The phylogenetic relationship of several oscillatorian *Cyanobacteria*, forming

blooms at Daecheong reservoirs, based on partial 16S rRNA gene sequences. *J. Microbiol. Biotechnol*. **11:** 504–507.
14. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem*. **153:** 375–380.
15. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, U.S.A.
16. Sanger, F., S. Nicklen, and A. R. Couslon. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:** 5463–5467.
17. Sneath. P. H. A., N. S. Mair, M. E. Sharpe, and J. G. Holt. 1986. *Bergey's Manual of Systematic Bacteriology*, pp. 1104-1138. Vol. 2. Williams and Wilkins Press, Baltimore, U.S.A.
18. Takai, Y., Y. Okumura, T. Tanaka, M. Sawada, S. Takahashi, M. Shiro, M. Kawamura, and T. Uchiyama. 1994. Binding characteristics of a new host family of cyclic oligosaccharides from inulin: Permethylated cycloinulo-hexose and cycloinulo-heptaose. *J. Org. Chem*. **59:** 2967–2975.
19. Yoo, J., K.-W. Han, H.-K. Kim, M.-H. Kim, and S.-T. Kwon. 2000. Purification and characterization of a thermostable β-glycosidase from *Thermus caldophilus* GK24. *J. Microbiol. Biotechnol.* **10:** 638–642.
20. Yoo, S. K., D. Kim, and D. F. Day. 2001. Co-production of dextran and mannitol by *Leuconostoc mesenteroides*. *J. Microbiol. Biotechnol*. **11:** 880–883.
21. Yoon, H. J., Y. J. Choi, O. Miyake, W. Hashimoto, K. Murata, and B. Mikami. 2001. Effect of His192 mutation on the activity of alginate lyase A1 from *Sphingomonas* species A1. *J. Microbiol. Biotechnol.* **11:** 118–123.