한국농화학회지 제 40 권 제 6 호(1997)
Agricultural Chemistry and Biotechnology
Vol. 40, No. 6, pp. 495~500(1997)

Molecular Cloning and Characterization of a Gene for Cyclodextrin Glycosyltransferase from *Bacillus* sp. E1

Jeongsik Yong,[1,2] Jin Nam Choi,[1,2] Sung Soon Park,[1,2] Cheon Seok Park,[1,3] Kwan Hwa Park,[1,3] and Yang Do Choi[1,2]*

[1]*Research Center for New Bio-Materials in Agriculture,* [2]*Department of Agricultural Chemistry,* [3]*Department of Food Science and Technology, Seoul National University, Sawon 441-744, Korea*

Abstract : To isolate a gene for cyclodextrin glycosyltransferase (CGTase) from alkalophilic *Bacillus* sp. E1, polymerase chain reaction (PCR) amplification was carried out. Direct molecular cloning of 1.2 kbp fragment and partial nucleotide sequence analysis of the PCR amplified clone, pH12, showed close homology with CGTases from *Bacillus* species. To investigate the genomic structure of the gene, Southern blot analysis of genomic DNA was carried out with the clone pH12 as a molecular probe. It showed that 5.3 kbp *Xba*I fragment was hybridized with the probe pH12. To isolate a genomic clone, genomic DNA library was constructed and a genomic clone for CGTase, pCGTE1, was isolated. Nucleotide sequence analysis of the clone pCGTE1 revealed that BCGTE1 contained 2,109 bp open reading frame encoding a polypeptide of 703 amino acids and showed over 94.3% amino acid sequence homology with CGTase of β-cyclodextrin producer, *Bacillus* sp. KC201.(Received October 7, 1997; accepted October 20, 1997)

Introduction

Cyclodextrins (CDs) are circular molecules made up of 6-8 glucose units via α-1,4-glycosidic linkage. Three types of CDs, α-, β-, and γ-CD (composed of six, seven, and eight glucose units, respectively), are synthesized from starch by cyclodextrin glycosyltransferase (CGTase; EC 2.4.1.19). These CDs solubilize or stabilize a variety of fine organic and inorganic compounds in water by incorporating within their hydrophobic cavities. Considering its solubility and the capacity to hold larger molecules of interest, β- and γ-CD are regarded as more desirable form than α-CD (Thoma and Stewart, 1965). CGTase is a starch degrading enzyme, and produces CDs from starch. CGTase is used as an important enzyme to make CDs in agricultural, food, pharmaceutical, and medical industries.

Genes coding for CGTase were cloned from various bacterial sources and expressed in heterologous or homologous host (Binder *et al.*, 1986; Takano *et al.*, 1986; Kimura *et al.*, 1987a; Kaneko *et al.*, 1988; Hill *et al.*, 1990; Nitschke *et al.*, 1990; Sin *et al.*, 1991; Fujiwara *et al.*, 1992b). The Genus *Bacillus* has been reported to be one of the best sources for CGTases (Takano *et al.*, 1986; Kimura *et al.*, 1987a; Kaneko *et al.*, 1988; Hill *et al.*, 1990; Nitschke *et al.*, 1990; Sin *et al.*, 1991; Fujiwara *et al.*, 1992b). Since CGTases from alkalophilic *Bacillus* species are more active and stable in wide range of pH and temperature than other microbial CGTases, they are more promising for industrial uses (Kimura *et al.*, 1987a,b; Kaneko *et al.*, 1988,1989; Kitamoto *et al.*, 1992). Molecular cloning of CGTase genes from alkalophilic *Bacillus* sp. producing β-CD predominantly suggested the molecular structure of CGTase and functional active sites (Kimura *et al.*, 1987a,b; Kaneko *et al.*, 1988; Nakamura *et al.*, 1992).

We isolated a gene for CGTase from alkalophilic *Bacillus* sp. E1 (BCGTE1) and determined its molecular structure in this study. BCGTE1 produces β- and γ- CD to the ratio of 7 : 1 rather than α-CD (Park *et al.*, 1992).

Materials and methods

Bacterial strains and culture media

Bacterial strains used in this experiment are *E. coli* MC1061 [F$^-$, *araD139*, Δ(*ara, leu*)7696, Δ(*lac*)Y74, *gal*U$^-$, *gal*K$^-$, *hsd*R$^-$, *str*A] for the transformation and plasmid conservation. *E. coli* MB406 [F$^-$, *hsd*R514 (rk$^-$, mk$^+$), *sup*E44, *sup*F58, *lac*Y1 or Δ(*lac*IZY)6, *gal*T22, *met*B1, *trp*R55, λ$^-$] was used for lambda EMBL3 host and

Key words : cyclodextrin, cyclodextrin glycosyltransferase, CGTase, *Bacillus* sp., molecular cloning, PCR
* Corresponding author

E. coli BL21 (DE3) [*hsd*S, *gal* (λcI^{ts857}, *ind*1, *Sam*7, *nin*5, *lac*UV5-T7 gene 1)] for the expression of recombinant BCGTE1. Alkalophilic *Bacillus* sp. E1 producing CGTase was screened out from soil (Park *et al.*, 1992).

All strains of *E. coli* were grown in LB medium (1% tryptone, 0.5% yeast extract and 0.5% NaCl). Transformed *E. coli* was cultured in LB medium with ampicillin (50 μg/m*l*). Alkalophilic *Bacillus* sp. E1 producing CGTase was cultured in Horikoshi's alkaline medium II consisted of 2% soluble starch, 0.5% polypeptone, 0.5% yeast extract, 0.1% K_2HPO_4, 0.02% $MgSO_4$ $7H_2O$ and 1% Na_2CO_3 (Kaneko *et al.*, 1988).

Recombinant DNA techniques and nucleotide sequencing

All cloning steps were carried out according to the procedure of Sambrook *et al.* (1989). Chromosomal DNA of *Bacillus* sp. E1 was purified by the method of Dubnau *et al.* (1971). Genomic library of *Bacillus* sp. E1 was constructed into EMBL3 (Sambrook *et al.*, 1989). Nucleotide sequencing was carried out by the dideoxynucleotide chain termination method of Sanger *et al.* (1977). Universal M13 primers for reverse and forward reaction were used and the reaction products were analyzed by 6 M urea-polyacrylamide gel electrophoresis.

Polymerase chain reaction

Polymerase chain reaction (PCR) was carried out by the procedure of Bej *et al*(1991). For PCR amplification of CGTase from *Bacillus* sp. E1, the upstream primer (5'-CCAACAAGCAGAATTTCAG-3') and the downstream primer (5'-TAATAGATGGCAGGCA CACCG-CGTGAAGTCA-3') were taken from conserved regions of CGTase from various *Bacillus* species.

Results and Discussion

A gene for CGTase was isolated from *Bacillus* sp. E1

To isolate a gene for CGTase from alkalophilic *Bacillus* sp. E1 (BCGTE1), PCR amplification was attempted. The primer sequences were based on the conserved sequences of CGTase genes from various *Bacillus* species (Fig. 1). Multiple nucleotide sequence comparison revealed highly conserved stretches of nucleotide (nt) sequences in genes for *Bacillus* CGTases at the position of +100~+120 nt and +1130~+1150 nt from the translational initiation site. The distance between two conserved regions was about 1,030 nts. PCR amplification gave a DNA fragment of about 1.2 kbp as expected. It was cloned (pH12) and utilized as a molecular probe to isolate a gene for CGTase.

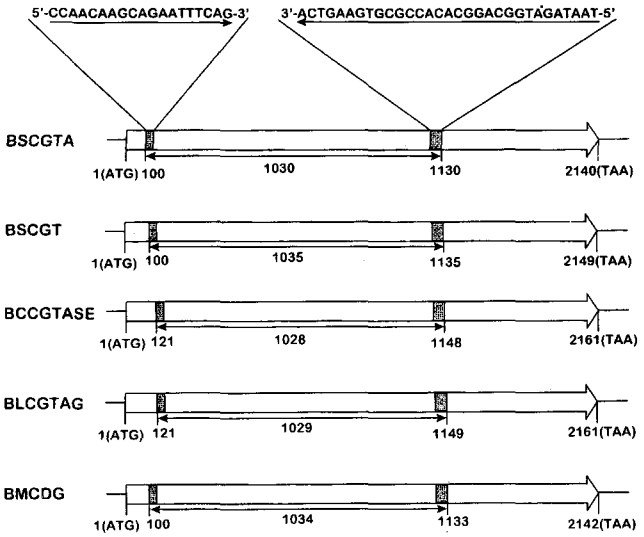

Fig. 1. Nucleotide sequences for PCR primers. Five CGTase genes were compared and two conserved regions were extracted. Nucleotide sequences for BSCGTA was taken from *Bacillus* sp. 1011 (Kimura *et al.*, 1987), BSCGTC from *Bacillus* sp. 38-2 (Kaneko *et al.*, 1988), BCCGTASE from *B. circulans* strain No. 8 (Nitschke *et al.*, 1990), BLCGTAG from *B. licheniformis (Hill et al.*, 1990), and BMCDG from *B. macerans* (Takano *et al.*, 1986).

To investigate the structure of a gene for CGTase in the *Bacillus* genome, Southern blot analysis was carried out with the PCR clone pH12 as a probe. It hybridized

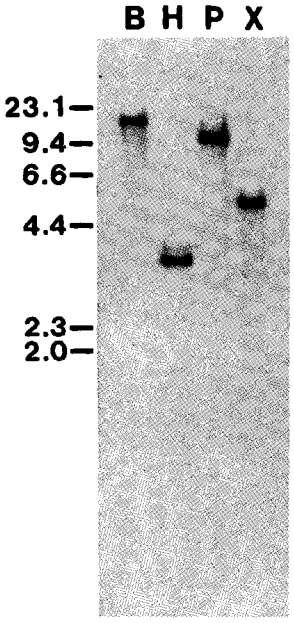

Fig. 2. Genomic Southern blot analysis of *Bacillus* sp. E1 for CGTase. Genomic DNA of *Bacillus* sp. E1 was digested with restriction enzyme *Bam*HI (lane B), *Hind*III (lane H), *Pst*I (lane P), or *Xba*I (lane X), separated by 0.8% agarose gel electrophoresis, and transferred onto nylon membrane. The blot was hybridized with the clone pH12 encoding BCGTE1 labeled by random primer extension.

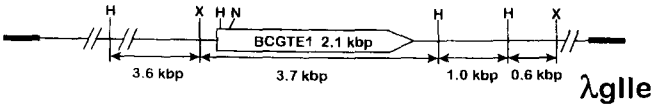

Fig. 3. The structure of the genomic clone λgIIe from *Bacillus* sp. E1. The *Xba*I fragment of 5.3 kbp was subcloned from λgIIe to give pCGTE1 for CGTase. Vector sequences are shown in thick lines. H, *Hind*III; X, *Xba*I; N, *Nco*I

with a 3.5 kbp *Hind*III and a 5.3 kbp *Xba*I fragment. Considering the average size of CGTase genes of about 2.5 kbp and simple pattern by genomic Southern blot, it is concluded that the gene for BCGTE1 is a single copy unique sequence (Fig. 2).

To isolate a full-length genomic clone encoding BCGTE1, genomic DNA library was constructed from *Bacillus* sp. E1 and positive recombinant phages were screened out by plaque hybridization with the clone pH 12 as a molecular probe. A recombinant clone λgIIe was isolated and the 5.3 kbp *Xba*I fragment was subcloned into pUC18 plasmid vector, which gave the pCGTE1. The structure and restriction enzyme map of the genomic clones λgIIe and pCGTE1 are shown in Fig. 3.

Nucleotide sequence of pCGTE1 was determined

To characterize the structure of pCGTE1, the nucleotide sequence was determined as was shown in Fig. 4. There was an open reading frame from the very beginning of the sequence, however, no usual translation initiation codon ATG was found near the N-terminal region. The translational initiation codon was assigned by the hydropathy analysis and the comparison with other CGTase genes (Data not shown). It could be UUG encoding leucine rather than AUG, as was reported in *B. ohbensis* (Sin *et al.*, 1991). It has been reported the GUG and UUG could be employed as a translational initiation codon even though the occurrence is rare and the translational efficiency is lower (Reddy *et al.*, 1985). Putative promoter sequences, TTTACG and TATTAA, homologous to the consensus sequences for RNA poly- merase of *B. subtilis* (Moran *et al.*, 1982) were found at the positions of -163 and -138 nt from the translational initiation codon, respectively. A possible ribosomal binding site, AGGAGG, was found at 13 nt upstream from the translational initiation codon as shown in Fig. 4 (McLaughlim *et al.*, 1981). An open reading frame from UUG at +1 to UAA at +2,110 encoding 703 amino acid residues was deduced. The molecular weight (Mr) of the nascent BCGTE1 was estimated to be 78,772. A palindromic sequence that could form a stable stem and loop structure (ΔG=-22.8 kcal/

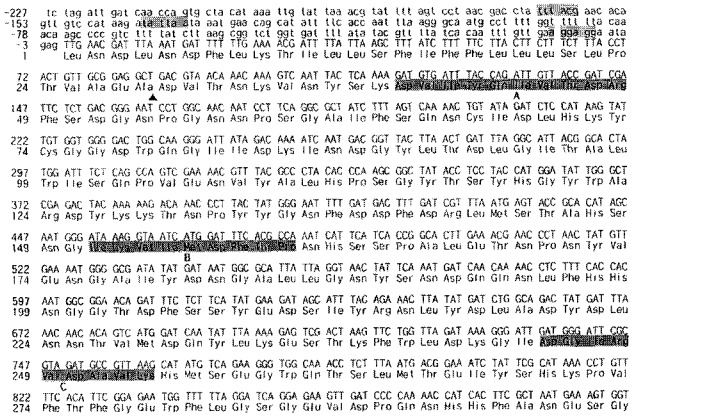

Fig. 4. Nucleotide and deduced amino acid sequences of the clone pCGTE1 encoding CGTase from *Bacillus* sp. E1. The putative -35 sequence (TTTACG), -10 sequences (TATTAA), and ribosome binding site (AGGAGG) are shown in shades. Five highly conserved regions of CGTase are shown in shade designated by A through E. Putative translation initiation codon (TTG) and processing site for the signal sequence (arrow head) was assigned by comparison with other CGTases from *Bacillus* species. Numbering of nucleotide and amino acid sequences start from the translational initiation site. The palindromic sequence at downstream is shown by horizontal arrows under the sequence. The nucleotide sequence data reported in this paper appears in the EMBL and GeneBank Nucleotide Sequence Databases with the accession number Z34466.

mol; nt +2,126 through +2,157) followed by an A+T-rich sequence characteristic of ρ-independent transcriptional terminator of *E. coli* was also noticed (Rosenberg *et al.*, 1979).

To study the primary structure of BCGTE1, it was compared with those of other CGTases from *Bacillus* species (Table 1). The deduced amino acid sequence of BCGTE1 showed 94.3% homology to that of alkalophilic *Bacillus* sp. KC201 (BKC201; Kitamoto *et al.*, 1992) and 79.1% to that of *B. ohbensis* (Sin *et al.*, 1991). Both of

Table 1. Relative homology (percent of identity) between amino acid sequences of various CGTase from *Bacillus* sp. and *Klebsiella pneumoniae*

	Percent of Identity									
	BCCGTAE[b]	BKC201[c]	BLCGTAG[d]	BMCDG[e]	BOCGTAA[f]	BSCGT1[g]	BSCGTA[h]	BSCGTC[i]	BSCGTDNA[j]	CGTKP[k]
BCGTE1[a]	55.0	94.0	56.2	52.4	79.2	59.0	56.3	55.9	55.8	35.9
BCCGTASE		52.5	90.5	63.1	58.6	60.6	70.4	69.5	97.1	36.4
BKC201			57.6	56.1	77.8	56.4	58.2	56.2	56.4	34.3
BLCGTAG				62.6	58.0	59.7	70.6	69.6	90.9	35.7
BMCDG					54.6	54.7	64.0	63.3	63.1	30.2
BOCGTAA						63.0	57.5	57.2	57.3	36.4
BSCGT1							60.9	60.1	61.5	29.5
BSCGTA								97.2	69.7	28.3
BSCGTC									68.8	29.3
BSCGTDNA										36.4

[a] from *Bacillus* sp. E1 (this study)
[b] from *B. circulans* straim No. 8 (Nitchke *et al.*, 1990)
[c] from *Bacillus* sp. KC201 (Kitamoto *et al.*, 1992)
[d] from *B. licheniformis* (Hill *et al.*, 1990)
[e] from *B. macerans* (Takano *et al.*, 1986)
[f] from *B. ohbensis* (Sin *et al.*, 1991)
[g] from *B. stearothermophilus* (Fujiwara *et al.*, 1992)
[h] from *Bacillup* sp. 1011 (Kimura *et al.*, 1988)
[i] from *Bacillup* sp. strain No. 38-2 (Kaneko *et al.*, 1988)
[j] from *Bacillup* sp. strain ACM-1.7.9.3.-D (Akhmetzjanov, unpublished)
[k] from *K. pneumoniae* (Bender *et al.*, 1986)

them are known to be β-CD producers as is BCGTE1 (Sin *et al.*, 1991; Kitamoto *et al.*, 1992; Park, *et al.*, 1992). Sequence homologies to CGTases of the other *Bacillus* species were below 60% (Table 1).

Five highly conserved regions of CGTases were noticed by the comparison of amino acid sequences between CGTases as shown in Fig. 4. Three of them, regions B, C and D, were also conserved in amylases, which were known to be the substrate binding site of starch hydrolysis enzymes (Kimura *et al.*, 1987b; Itkor *et al.*, 1990). The other regions, A and E, were believed to be involved in transglycosylation reaction of CGTase for the cyclization of maltooligosaccharides (Kaneko *et al.*, 1989,1990; Kimura *et al.*, 1989; Fujiwara *et al.*, 1992a). Cyclization (transglycosylation) activity is unique to CGTase among other starch hydrolysis enzymes. It was reported that the deletion of the N-terminal or C-terminal region in CGTase affected not only the transglycosylation activity but also the stability of the enzyme (Kaneko *et al.*, 1989, 1990; Kimura *et al.*, 1989; Fujiwara *et al.*, 1992a). These results strongly support the involvement of region A and E in unique cyclization activity of CGTase.

It has been known that CGTase is an extracellular enzyme in *Bacillus* species except CGTase of *B. megaterium* (Ueda and Nagai, 1988). The N-terminal signal sequence of the nascent polypeptide is responsible for the vectorial transport of CGTase into the culture media. According to the method of von Heijne (1986) predicting for the signal sequence cleavage site, a peptide bond between alanine and aspartate at amino acid residue 29 and 30 was expected to be cleaved in nascent BCGTE1 by post-translational processing. The Mr of the signal sequence of BCGTE1 was calculated to be 3,281 and the mature BCGTE1 to be 75,490.

Usually CGTases produced from recombinant *E. coli* have the same enzymatic properties as parental CGTases (Binder *et al.*, 1986; Takano *et al.*, 1986; Nitschke *et al.*, 1990; Sin *et al.*, 1991; Lee *et al.*, 1994). Modification and overexpression of the gene for BCGTE1 by the recombinant DNA technology may provide a chance to study the post-translational processing and vectorial transport mechanism of the protein.

Acknowledgements

The present investigation was supported by a grant from the Research Center for New Bio-Materials in Agriculture designated by the Korea Science and Engineering Foundation and in part by a grant from the Lotte Scholarship Foundation.

References

1. Thoma, J. A. and L. Stewart (1965) In : Starch Chemistry and Technology, vol. 1, Academic Press Inc., New York, pp 109-145
2. Binder, F., O. Huber and A. Bock (1986) Cyclodextrin glycosyltransferase from *Klebsiella pneumoniae* M5a1 : cloning nucloetide sequence and expression. *Gene* **47** : 269-277
3. Takano, T., M. Fukuda, M. Monma, S. Kobayashi, K. Kainuma and K. Yamane (1986) Molecular cloning, DNA nucleotide sequencing, and expression in *Bacillus subtilis* cells of the *Bacillus* macerans cyclodextrin glucanotransferase gene. *J. Bacteriol.* **166** : 1118-1122
4. Kimura, K., T. Takano and K. Yamane (1987a) Mole-

cular cloning of β-cyclodextrin synthetase gene from an alkalophilic *Bacillus* and its expression in *Escherichia coli* and *Bacillus subtilis*. *Appl. Microbiol. Biotechnol.* **26**: 149-153

5. Kaneko, T., T. Hamamoto and K. Horikoshi (1988) Molecular cloning and nucleotide sequence of the cyclomaltodextrin glucanotransferase gene from the alkalophilic *Bacillus* sp. strain no. 38-2. *J. Gen. Microbiol.* **134**: 97-105
6. Hill, D. E., R. Aldape and J. D. Rozzell (1990) Nucleotide sequence of a cyclodextrin glycosyltransferase gene, cgtA, from *Bacillus licheniformis*. *Nucleic Acids Res.* **18**: 199
7. Nitschke, L., K. Heeger, H. Bender and G. E. Schulz (1990) Molecular cloning, nucleotide sequence and expression in *Escherichia coli* of the β-cyclodextrin glycosyltransferase gene from *Bacillus circulans* strain no. 8. *Appl. Microbiol. Biotechnol.* **33**: 542-546
8. Sin, K. A., A. Nakamura, K. Kobayashi, H. Masaki and T. Uozumi (1991) Cloning and sequencing of a cyclodextrin glucanotransferase gene from *Bacillus ohbensis* and its expression in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **35**: 600-605.
9. Fujiwara, S., H. Kakihara, K. Sakaguchi and T. Imanaka (1992a) Analysis of mutations in cyclodextrin glucanotransferase from *Bacillus stearothermophilus* which affect cyclization characteristics and thermostability. *J. Bacteriol.* **174**: 7478-7481
10. Kimura, K., S. Kataoka, Y. Ishii, T. Takano and K. Yamane (1987b) Nucleotide sequence of the β-cyclodextrin glucanotransferase gene of alkalophilic *Bacillus* sp. strain 1011 and similarity of its amino acid sequence to those of α-amylases. *J. Bacteriol.* **169**: 4399-4402
11. Kaneko, T., K. B. Song, T. Hamamoto, T. Kudo and K. Horikoshi (1989) Construction of chimeric series of *Bacillus* cyclodextrin glucanotransferase and analysis of the thermal stabilities and pH optima of the enzymes. *J. Gen. Microbiol.* **135**: 3447-3457
12. Kitamoto, N., K. Kimura, Y. Kito and K. Ohmiya (1992) Cloning and sequencing of the gene encoding cyclodextrin glucanotransferase from *Bacillus* sp. KC201. *J. Fermen. Bioeng.* **74**: 345-351
13. Nakamura, A., K. Haga, S. Ogawa, K. Kuwano, K. Kimura and K. Yamane (1992) Functional relationships between cyclodextrin glucanotransferase from an alkalophilic *Bacillus* and α-amylases. *FEBS Lett.* **296**: 37-40
14. Park, C. S., E. J. Woo, S. U. Kuk, B. C. Seo, K. H. Park, and H. Lim (1992) Purification and characterization of cyclodextrin glucanotransferase from *Bacillus* sp. E1. *Kor. J. Microbiol. Biotechnol.* **20**: 156-163
15. Sambrook, J., E. F. Fritsch and T. Maniatis (1989) Molecular cloning : A laboratory manual, 2nd ed, Cold Spring Harbor Laboratory Press
16. Dubnau, D. and R. D. Abenson (1971) Fate of transforming DNA following uptake by competent *Bacillus subtilis*. *J. Mol. Biol.* **56**: 209-221
17. Sanger, F., S. Nicklen and A. R. Coulson (1977) DNA sequencing with chain terminating inhibitors. *Proc. Nat'l. Acad. Sci. USA.* **74**: 5463
18. Bej, A. K., M. H. Mahbubani and R. M. Atlas (1991) Amplification of nucleic acids by polymerase chain reaction (PCR) and other methods and their applications. *Critical Reviews Biochemistry and Molecular Biology* **26**: 301-334
19. Moran, C. P., N. Lang, S. F. LeGtice, G. Lee, M. Stephens, A. L. Sonenshein, J. Pero and R. Losick (1982) Nucleotide sequence that signal the initiation of transcription and translation in *Bacillus subtilis*. *Mol. Gen. Genet.* **186**: 339-346
20. McLaughlim, J. R., C. L. Murray and J. C. Rabinowits (1981) Unique features in the ribosome binding site sequence of the Gram-positive *Staphylococcus aureus* β -lactamase gene. *J. Biol. Chem.* **256**: 11283-11291.
21. Rosenberg, M. and D. Court (1979) Regulatory sequences involved in the promotion and termination of RNA transcription. *Annu. Rev. Genet.* **13**: 319-353
22. Itkor, P., N. Tsukagoshi and S. Udaka (1990) Nucleotide sequence of the raw-starch-digesting amylase gene from *Bacillus* sp. B1018 and its strong homology to the cyclodextrin glucanotransferase genes. *Biochem. Biophys. Res. Commun.* **166**: 630-636
23. Kaneko, T., T. Kudo and K. Horikoshi (1990) Comparison of CD composition by chimeric CGTase. *Agric. Biol. Chem.* **54**: 197-201
24. Fujiwara, S., H. Kakihara, B. W. Kim, A. Lejeune, M. Kanemoto, K. Sakaguchi and T. Imanaka (1992b) Cyclization characteristics of cyclodextrin glucanotransferase are conferred by the NH_2-terminal region of the enzyme. *Appl. Environ. Microbiol.* **58**: 4016-4025
25. Ueda, H. and T. Nagai (1988) In the Book of Abstracts of 4th Int. Symp. on Cyclodextrins. Munich, FRG
26. von Heijne, G. (1986) A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* **14**: 4383-4690
27. Lee, K. C. P. and B. Y. Tao, (1994) High-level expression of cyclodextrin glycosyltransferase in *E. coli* using a T7 promoter expression system. *Starch* **46**: 67-74
28. Reddy, P., A. Peterkofsky and K. Mckenny (1985) Translational efficiency of the *E. coli* adenylate cyclase gene : mutating the UUG initiation codon to GUG or AUG results in increased gene expression. *Proc. Nat'l. Acad. Sci. USA.* **82**: 5656-5660
29. Kimura, K., S. Kataoka, A. Nakamura, T. Takano, S. Kobayashi and K. Yamane (1989) Functions of the COOH-terminal region of cyclodextrin glucanotransferase of alkalophilic *Bacillus* sp. #1011 : relation to catalyzing activity and pH stability. *Biochem. Biophys. Res. Commun.* **161**: 1273-1279

Bacillus sp. E1 의 cyclodextrin 생산효소 유전자 분리 및 구명

용정식[1,2] · 최진남[1,2] · 박성순[1,2] · 박천석[1,3] · 박관화[1,3] · 최양도[1,2]* ([1]서울대학교 농업생물신소재 연구센터, [2]농화학과, [3]식품공학과)

초 록 : Cyclodextrin을 합성하는 효소 CGTase를 호염기성 *Bacillus* sp. E1으로부터 분리하기 위하여 PCR을 실시하였다. PCR을 위하여 합성한 primer의 염기서열은 현재까지 보고된 CGTase 유전자의 염기서열을 비교 분석하여 가장 높게 보존된 영역을 찾아내어 선택하였다. PCR 증폭 결과 1.2 kbp 크기의 DNA 절편을 얻을 수 있었고 이를 molecular probe로 이용하여 Southern blot 분석을 실시하였다. Southern blot 분석결과 CGTase 유전자는 염색체 DNA를 제한효소 *Xba*I으로 절단한 5.3 kbp 절편내에 존재한다는 사실을 알아내었다. CGTase 유전자를 분리하기 위하여 유전자 은행을 제조한 후 선별작업을 실시하여 genomic clone인 pCGTE1을 얻을 수 있었다. pCGTE1의 염기서열을 결정한 결과 분리한 CGTase 유전자는 2109 bp의 open reading frame을 가지며 이는 703개의 아미노산으로 구성된 단백질을 coding하는 것으로 나타났다. 아미노산 서열의 유사성을 비교한 결과 *Bacillus* sp. KC201의 CGTase 와 가장 높은 94.3% 동질성을 나타내었다.

찾는말 : cyclodextrin, cyclodextrin glycosyltransferase, CGTase, *Bacillus* sp., molecular cloning, PCR
*연락저자