Short Communication

Cloning and Expression of the *Bacillus subtilis* No. 313 γ -Cyclodextrin Forming CGTase Gene in *Escherichia* *coli*

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Received April 17, 1986

Cyclomaltodextrin glucanotransferase (1,4- α -D-glucan-4- α -D-(1,4- α -D-glucano)-transferase (cyclizing), EC 2.4.1.19, CGTase) catalyzes the formation of cyclodextrins (CD) from starch and some carbohydrates.¹⁾

CGTases are classified into two types, that is, *B. macerans* type enzymes which produce α -CD from starch²⁾ and alkalophilic *Bacillus* type enzymes which produce β -CD from starch.³⁾ No enzymes which produce γ -CD predominantly have been discovered yet. Recently we isolated a new bacterium (*B. subtilis* No. 313) that produces a CGTase that forms γ -CD predominantly γ -CGTase.⁴⁾ In this paper we describe the cloning of the gene for the γ -CGTase of *B. subtilis* No. 313 in *E. coli*, using pBR322.

B. subtilis No. 313, which produces γ -CGTase, *E. coli* K-12 HB101 (*pro lueB B1 lacY hsdR hsdM ara-14 galKz xyl-5 mtl-1 supE44 F⁻ endA recA Str^r*) and the plasmid, pBR322 (Ap^r Tc^r), were used throughout.

To obtain the DNA fragment containing the γ -CGTase gene, we constructed a recombinant plasmid library that was representative of the genome of *B. subtilis* No. 313. Total genomic DNA from *B. subtilis* No. 313 was partially digested with restriction endonuclease *Sau*3AI, and fragments larger than 2 kilobase pairs (kb) were collected by sucrose gradient centrifugation. The *Sau*3AI-digested DNA fragments were inserted into the *Bam*HI site of

pBR322. This ligated DNA mixture was used for the transformation of *E. coli* HB101.

The γ -CGTase activity could be detected directly on LB agar plates containing 0.2% starch, because a clear zone was observed around a colony producing γ -CGTase on spraying with an I₂ solution. Out of 10,000 Ap^r Tc^s transformants, four exhibited clear zones around their colonies. The Ap^r, γ -CGTase⁺ transformants harboured the hybrid recombinant plasmid, pMT1, which contained the 3.4kb *Sau*3AI fragment. The restriction map of plasmid pMT1 is presented in Fig. 1. To determine which fragment was necessary for the expression of γ -CGTase, a *Pvu*II-*Aat*I fragment was inserted into the *Bam*HI site of pBR322 by means of *Bam*HI linker (pMT2). The plasmid, pMT2, was introduced into *E. coli* HB101, and then γ -CGTase activity was assayed. γ -CGTase activity was detected in *E. coli* carrying pMT2 (140 u/ml, as dextrinizing activity). It was concluded that the 2.8 kb *Pvu*II-*Aat*I fragment contained the γ -CGTase gene (Fig. 1).

FIG. 1. Structures of Plasmids pMT1 and pMT2.

The thin line represents pBR322 and the thick line the *B. subtilis* No. 313 DNA. The restriction sites for *Aat*I(AI), *Aat*II(AII), *Bam*HI(B), *Eco*RI(E), *Hinc*II(Hc), *Hind*III(Hd), *Pst*I(Ps), *Pvu*II(Pv), *Sac*II(SII) and the *Bam*HI-*Sau*3AI boundary (B/S) are indicated.

FIG. 2. High Performance Liquid Chromatogram of Enzymatic Products of pMT2 Encoded γ -CGTase.

A, standard mixture of α -, β -, γ -CD and glucose; B, the enzymatic products of pMT2 encoded γ -CGTase.

To confirm the origin of the DNA inserted in the pBR322 plasmid, we hybridized biotin-labeled pMT1 with the restriction enzyme digested chromosomal DNAs of *B. subtilis* No. 313 and *E. coli*, which had been immobilized on Durapore filters (Millipore Co.). The hybridized DNAs were detected with a BRL DNA detection system (Bethesda Research Laboratories, U.S.A.). Biotin-labeled pMT1 hybridized with the restriction fragment of unlabeled pMT1, and also hybridized with a 2.8 kb DNA fragment from *B. subtilis* No. 313. No complementary sequences to pMT1 were detected in *E. coli*.

The enzymatic products of pMT2-encoded γ -CGTase were analyzed by performance liquid chromatography and the BCG

FIG. 3. Differential Absorption Spectra of BCG Complexes.

One ml of γ -CD and the enzymatic products of pMT2 encoded γ -CGTase was mixed with 0.1 ml of 5 mM BCG and 2 ml of 0.2 M citrate buffer (pH 4.2), and then the differential absorbance spectra were measured. (---) γ -CD and (—) the enzymatic products of pMT2 encoded γ -CGTase.

method⁵⁾ (Figs. 2 and 3). The plasmid encoded γ -CGTase in pMT2 produced only γ -CD from starch, *i.e.*, neither α - nor β -CD could be detected so far as tested.

These results indicated that the cloned DNA fragment contained the structural gene for the *B. subtilis* No. 313 γ -CGTase.

REFERENCES

- 1) D. French, *Carbohydr. Chem.*, **12**, 189 (1957).
- 2) S. Kobayashi, K. Kainuma and S. Suzuki, *Carbohydr. Res.*, **61**, 229 (1978).
- 3) N. Nakamura and K. Horikoshi, *Agric. Biol. Chem.*, **40**, 753 (1976).
- 4) T. Kato and K. Horikoshi, in preparation.
- 5) T. Kato and K. Horikoshi, *Anal. Chem.*, **56**, 1738 (1984).