

Characterization of restriction endonuclease *Cbi*I, an isoschizomer of *Asu*II, from *Clostridium bifermentans* strain B-4

Makoto Murakami, Osamu Ozawa, Tadashi Kanematsu and Yuzo Yamada[1]
Research Department, Nissin Sugar Mfg. Co., Ltd, 4-9-11 Toyosu, Kohto-ku, Tokyo 135 and [1]Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Shizuoka University, 836 Ohya, Shizuoka 422, Japan

Submitted May 2, 1991

We have found that an extremely large amount of a restriction endonuclease, designated *Cbi*I, is produced within the cells of *Clostridium bifermentans* strain B-4.

*Cbi*I was purified from cell extracts by combined high performance liquid chromatographies on DEAE-Toyopearlpak 650M, TSKgel DEAE-5PW, TSKgel HA-1000 and TSKgel G3000SW (1). The purified enzyme (0.4 mg protein from 1.7 g cell extract) was homogeneous on polyacrylamide gel disc electrophoresis (PAGDE). The relative molecular mass of the enzyme was calculated as 49,000 daltons by gel filtration and by SDS-PAGDE. These data indicated that *Cbi*I has a monomeric structure.

The enzyme worked best at 37°C and pH 7.0. The enzyme worked in the wide pH range (pH 5.0−8.0) and in the wide range of NaCl concentrations (0−150 mM). The enzyme was stable up to 55°C against the preincubation at pH 7.5 for 5 min and between pH 4.0 and 7.5 against the preincubation at 4°C for 24 hr.

The enzyme cleaved lambda, M.*Tth* HB8I-methylated (5′-T-CGA^{CH3}−3′) lambda, Ad2, M13mp18 RF I, φX174 RF I and pBR322 DNAs at 7, 0, 1, 0, 0 and 0 site, respectively (Figure 1). The digestion patterns of Ad2 DNA by the purified enzyme were not disturbed on double digestion with *Nsp* (7524)V (isoschizomer of *Asu*II). Lambda DNA was digested with the enzyme and labeled at the 5′-termini with ^{32}P. The labeled molecules were treated with *Eco*0109I. The resulting two DNA fragments with 353 and 817 base pairs were sequenced by the method of Maxam and Gilbert (2). The smaller fragment revealed gel patterns of 5′-C-G-A-A-G-G-A-A-A-G---- from coordinate 27,982 in the 3′-direction (coordinate 27,983 and further) (Figure 2). The gel patterns of the larger fragment were 5′-C-G-A-A-T-T-G-A-A-G----from coordinate 29,153 in the 3′-direction (coordinate 29,152 and further). The 5′-terminal nucleotides were identified as C for both fragments by the procedure of Ikawa *et al.* (3).

From the results obtained above, the restriction endonuclease from *C.bifermentans* strain B-4 recognizes and cleaves the following sequence,

5′-T-T/C-G-A-A-′3
3′-A-A-G-C/T-T-′5.

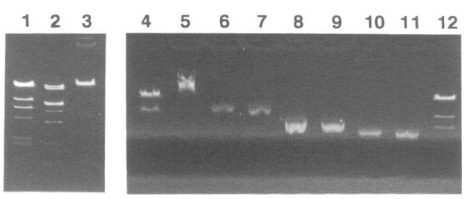

Figure 1. *Cbi*I digests; lane 1, 12: lambda-*Hin*dIII, lane 2: lambda-*Cbi*I, lane 3: M.*Tth*HB8I-methylated (5′-TCGA^{CH3}−3′) lambda-*Cbi*I, lane 4: Ad2-*Cbi*I, lane 5: Ad2, lane 6: M13mp18 RF-*Cbi*I, lane 7: M13mp18 RF, lane 8: φX174 RF-*Cbi*I, lane 9, φX174 RF, lane 10: pBR322-*Cbi*I, lane 11: pBR322.

ACKNOWLEDGEMENT

We wish to thank Professor Emeritus Dr T.Mitsuoka, Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Tokyo, Japan, for supplying *C.bifermentans* strain B-4.

REFERENCES

1. Murakami,M., Ozawa,O., Kanematsu,T. and Yamada,Y. (1991) *Agric. Biol. Chem.* **55**, 261−263.
2. Maxam,A.M. and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560−564.
3. Ikawa,S., Shibata,T. and Ando,T. (1979) *Agric. Biol. Chem.* **43**, 873−875.

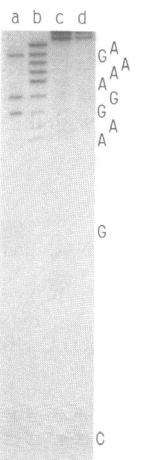

Figure 2. Determination of *Cbi*I-cleavage sites on DNA molecule. The ladders a, b, c and d represent reaction products of G, A+G, T+C and C, respectively.