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Isolation and characterization of restriction endonuclease *Bst*YI from *Bacillus stearothermophilus* Y406

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*Bst*YI, an isoschizomer of *Xho*II and *Mfl*I, has been purified from *Bacillus stearothermophilus* Y406. This enzyme recognized 5′...Pu/GATCPy...3′ in DNA and cleaved between Pu and G in this sequence. *Bst*YI can be easily isolated and purified by heparin-agarose column chromatography in a high yield (8000 units *Bst*YI can be obtained per g wet wt of cells).

Restriction endonuclease; Isoschizomer; *(Bacillus stearothermophilus)*

1. INTRODUCTION

Recently a great many nucleotide sequence-specific restriction enzymes have been isolated from a wide variety of microorganisms. The type II restriction endonucleases are tools indispensable to molecular biologists for studying the structure and function of DNA.

There are only two published restriction endonucleases of which the recognition sequence and cleavage site is 5′...Pu/GATCPy...3′. One is *Xho*II from *Xanthomonas holcicola* ATCC13461 [1], the other being *Mfl*I from *Microbacterium flavum* IAM1642 [2]. The yields of both *Xho*II in *X. holcicola* and *Mfl*I in *M. flavum* are very low and *Xho*II is difficult to prepare free of *Xho*I, so these two enzymes cannot be used extensively for cloning and sequencing of DNA.

Recently we have isolated *Bst*YI, another isoschizomer of *Xho*II, from *Bacillus stearothermophilus* Y406. We report here the isolation and some properties of this enzyme.

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2. MATERIALS AND METHODS

2.1. *Strain and growth condition*

B. stearothermophilus Y406 isolated from soil was grown in LB medium supplemented with 1.05 mM nitrilotriacetate, 0.59 mM $MgSO_4 \cdot 7H_2O$, 0.91 mM $CaCl_2 \cdot 2H_2O$ and 0.04 mM $FeSO_4 \cdot 7H_2O$ [3] to stationary phase at 60°C. 6 g wet cells were harvested by centrifugation from 1 l culture.

2.2. *Chemicals*

The following chemicals were used: λ DNA, λ DNA (N^6-methyladenine-free), pBR322 DNA and restriction endonuclease *Xho*II from New England BioLabs; Sephadex G-100 from Pharmacia; heparin-agarose from Shanghai Institute of Materia Medica, Chinese Academy of Sciences; M13 sequencing system kit from BRL and [α-^{32}P]dATP from Amersham.

2.3. *Assay buffer of* Bst*YI*

Assay buffer of *Bst*YI contained 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 100 μg/ml bovine serum albumin.

3. RESULTS AND DISCUSSION

3.1. *Preparation of* Bst*YI*

6 g wet cells were suspended in 30 ml PEMN buffer [10 mM K_2HPO_4-KH_2PO_4 (pH 7.4), 1 mM EDTA, 7 mM 2-mercaptoethanol, 1 mM NaN_3] and disrupted by sonication. After centrifugation at 100000 × *g* for 1 h, 5 M NaCl and freshly

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prepared streptomycin sulfate (10%) was added to the supernatant to a final concentration of 0.045 M and 1.5%, respectively. The nucleic acid precipitate was removed by centrifugation at 50000 × *g* for 1 h. After concentration against polyethylene glycol 6000, the concentrated sample (about 5 ml) was loaded onto a column (15 × 600 mm) of Sephadex G-100 equilibrated with PEMN buffer. Restriction endonuclease activity was eluted with the same buffer. Active fractions were pooled and loaded onto a column (10 × 70 mm) of heparin-agarose equilibrated with PEMN buffer containing 0.2 M NaCl, and the restriction endonuclease was eluted with a linear gradient of NaCl (0.2–1.0 M) in PEMN buffer. Enzyme activity was eluted at 0.5 M NaCl. The fraction with site-specific enzyme activity was concentrated against 50% glycerol and stored at −20°C. 50000 U *Bst*YI can be obtained from 6 g wet wt of cells (1 unit is defined as the amount of enzyme required to digest completely 1 μg λ DNA into DNA fragments in 1 h at 60°C).

3.2. *Characterization of* Bst*YI*

The cleavage patterns of *Bst*YI on λ DNA and pBR322 DNA were determined by agarose-ethidium bromide gel electrophoresis [4]. The results show that *Bst*YI possesses the same recognition sequence as that of *Xho*II (fig.1). The recognition sequence and cleavage site of *Bst*YI on M13mp19 were determined using the dideoxynucleotide chain-termination method [5]. Sequencing data show that the cleavage site of *Bst*YI is 5′...Pu/GATCPy...3′, the same as *Xho*II (fig.2).

*Bst*YI required Mg^{2+} as the only cofactor. The enzyme was active over the temperature range 37–65°C with maximum activity between 55 and 60°C. *Bst*YI efficiently cleaved DNA at low salt concentration (0 mM NaCl). The optimal pH of the assay buffer for optimum activity of *Bst*YI was 8.0. *Bst*YI was very stable under incubation at 50°C for as long as 8 h.

Although the recognition sequence and cleavage site of *Bst*YI are precisely the same as those of

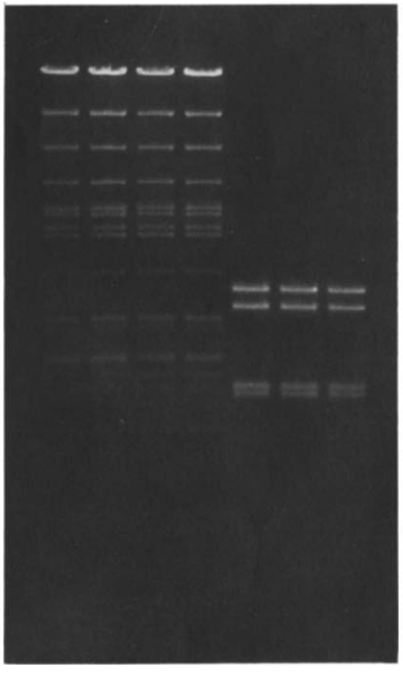

Fig.1. Cleavage patterns of restriction endonuclease *Bst*YI (lane 1) with λ DNA, *Bst*YI (lane 2), *Bst*YI + *Xho*II (lane 3), and *Xho*II (lane 4) with λ unmethylated DNA as the substrate. *Bst*YI (lane 5), *Bst*YI + *Xho*II (lane 6) and *Xho*II (lane 7) on agarose gel with pBR322 DNA as the substrate.

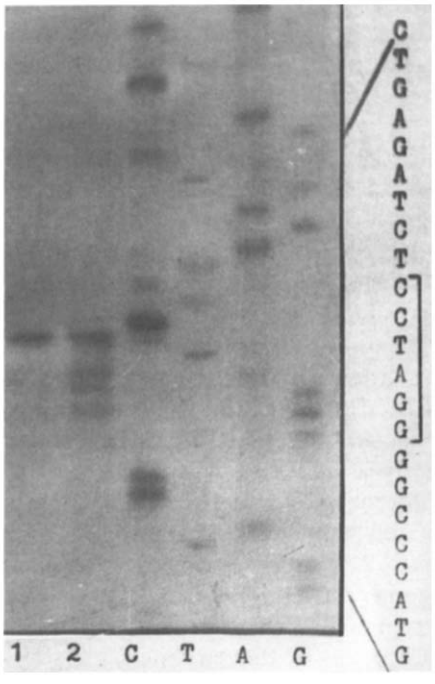

Fig.2. Determination of the recognition sequence and cleavage site of *Bst*YI on M13mp19 by the dideoxynucleotide chain-termination method. The complementary strand of M13 was labeled with [α-^{32}P]dATP. Lanes: 1, digestion with *Bst*YI; 2, incubation at 68°C for 20 min to inactivate partially the Klenow fragment, followed by digestion with *Bst*YI.

*Xho*II and *Mfl*I, *Bst*YI differs from *Mfl*I in that *Bst*YI digests both modified and unmodified DNA (fig.1) while *Mfl*I only cleaves the unmodified DNA.

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