Isolation and characterization of restriction endonuclease BstYI from *Bacillus stearothermophilus* Y406

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BstYI, an isoschizomer of XhoII and Mfl, 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. BstYI can be easily isolated and purified by heparin-agarose column chromatography in a high yield (8000 units BsrYI 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 XhoII from *Xanthomonas hollcicola* ATCC13461 [1], the other being Mfl from *Microbacterium flavum* IAM [2]. The yields of both XhoII in *X. hollcicola* and Mfl in *M. flavum* are very low and XhoII is difficult to prepare free of Xhol, so these two enzymes cannot be used extensively for cloning and sequencing of DNA.

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

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 MgSO₄·7H₂O, 0.91 mM CaCl₂·2H₂O and 0.04 mM FeSO₄·7H₂O [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 (N⁶-methyladenine-free), pBR322 DNA and restriction endonuclease XhoII 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-³²P]dATP from Amersham.

2.3. Assay buffer of BstYI

Assay buffer of BstYI contained 10 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 100 μg/ml bovine serum albumin.

3. RESULTS AND DISCUSSION

3.1. Preparation of BstYI

6 g wet cells were suspended in 30 ml PEMN buffer [10 mM K₂HPO₄-KH₂PO₄ (pH 7.4), 1 mM EDTA, 7 mM 2-mercaptoethanol, 1 mM NaN₃] and disrupted by sonication. After centrifugation at 100 000 × g for 1 h, 5 M NaCl and freshly
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 BstYI 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 BstYI

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

BstYI required Mg²⁺ as the only cofactor. The enzyme was active over the temperature range 37–65°C with maximum activity between 55 and 60°C. BstYI efficiently cleaved DNA at low salt concentration (0 mM NaCl). The optimal pH of the assay buffer for optimum activity of BstYI was 8.0. BstYI was very stable under incubation at 50°C for as long as 8 h.

Although the recognition sequence and cleavage site of BstYI are precisely the same as those of

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

Fig.2. Determination of the recognition sequence and cleavage site of BstYI on M13mp19 by the dideoxynucleotide chain-termination method. The complementary strand of M13 was labeled with [α-³²P]dATP. Lanes: 1, digestion with BstYI; 2, incubation at 68°C for 20 min to inactivate partially the Klenow fragment, followed by digestion with BstYI.
XhoI and MfI, BstYI differs from MfI in that BstYI digests both modified and unmodified DNA (fig.1) while MfI only cleaves the unmodified DNA.

REFERENCES