***Bsp*KT6I, a new site-specific endonuclease which cleaves the GATC site producing two nucleotide 5'-protruding ends**

Nina I.Shapovalova, Ludmila A.Zheleznaja¹ and Nicholas I.Matvienko*

Institute of Protein Research and ¹Institute of Theoretical and Applied Biophysics, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia

Received October 29, 1993; Accepted November 5, 1993

A new class II restriction endonuclease *Bsp*KT6I was isolated from soil thermophilic bacteria *Bacillus species* KT6. The enzyme was purified by chromatography on blue-agarose, heparine-Sepharose and hydroxylapatite. Digestion of the T7 DNA has revealed that the enzyme is an isomer of *Sau*3AI. Cleavage points were determined by the primed-synthesis reaction (1) using the M13mp18 DNA. The cleavage product resulted in the band comigrated with G (Fig. 1). Upon addition of the DNA-polymerase, a band appeared two nucleotides higher. These results indicate that the enzyme recognizes the palindromic sequence G[↓]ATC and cleaves it as indicated by the arrow creating two nucleotide 5'-extensions. It is the first isomer of *Sau*3AI producing such ends. The DNA fragments produced by this enzyme can be ligated with *Pvu*I-produced DNA fragments. The *Bsp*KT6I is sensitive to dam-methylation. It does not cleave the pBR322 isolated from the *E.coli* dam⁺ strain and results in almost complete digestion of pBR322 isolated from the dam⁻ strain (Fig. 2). The optimal reaction buffer is 10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂.

REFERENCE

1. Brown,N.L. and Smith,M. (1980) *Methods Enzymol.* **65**, 391-404.

Figure 1. Determination of the *Bsp*KT6I cleavage site. R: The template of primed-synthesis reaction was cleaved with the *Bsp*KT6I; P: The product from line R was treated with DNA-polymerase; G, A, C, T: The sequence ladder through the *Bsp*KT6I recognition sequence, using termination method.

Figure 2. Sensitivity of the *Bsp*KT6I to dam-methylation of DNA. (1) pBR322 from *E.coli* dam⁺; (2) pBR322 from *E.coli* dam⁻; (3) pBR322 from *E.coli* dam⁺ treated with the *Bsp*KT6I; (4) Molecular length markers in bp: T7 DNA treated with the *Bli* 736I; (5) pBR322 from *E.coli* dam⁻ treated with the *Bsp*KT6I.

* To whom correspondence should be addressed