Isolation and characterization of a restriction enzyme *BspO4I* from an alkalophilic bacterium

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A type II restriction enzyme, *BspO4I*, has been isolated from *Bacillus sp* O-4 (ATCC21536), an alkalophilic bacterium. The enzyme was an isoschizomer of *PvuII*, recognizing the six base palindromic sequence of 5'CAGCTG3' and cleaves between G and C residues to produce a blunt-ended cleavage products.

BspO4I was partially purified from a cell-free extract using column chromatographies on DEAE-cellulose, heparin-cellulofine and phosphocellulose. Optimal conditions for the enzyme activity were pH 8.5, 20 mM MgCl₂, 250 mM monovalent cation (NaCl or KCl), 10 mM 2-mercaptoethanol at 40°C. Some of these conditions including optima for monovalent and divalent cations differ from standard conditions for *PvuII* (1).

BspO4I cleaves pBR322, pUC19, ColE1 and M13mp19RF DNA at unique sites whose localizations corresponded to cleavage sites of *PvuII* (Figure 1. lanes 2, 3, 6, 7, 10, 11, 14, 15). A double digestion with *BspO4I* and *PvuII* suggested that these two enzymes cleave at the same site in the DNA (Figure 1. lanes 4, 8, 12, 16). Furthermore PBR322 DNA digested with *BspO4I* could be ligated by T4 DNA ligase, but could not be ligated by *E.coli* DNA ligase (data not shown). All of these observations indicate that *BspO4I* is an isoschizomer of *PvuII*.

The cleavage site of *BspO4I* was determined by primer extension experiments. The pBR322 DNA, which has one cleavage site for *BspO4I*, was digested by the enzyme, annealed with primers and then extended with Klenow fragment. The primer extension reactions were performed with both forward and reverse primers. Dideoxy sequencing reactions were also carried out using the same primers and electrophoresed in parallel with the extended products. The results showed that the extended products from both primers comigrate with the third G in the sequence 5'CAGCTG3' (Figure 2. lanes F and R). These data indicate that *BspO4I* recognizes and cleaves the following sequence:

REFERENCE

- Nasri, M., Sayadi, S. and Thomas, D. (1985) *FEBS Lett.* **185**, 101-104.

Figure 1. *BspO4I* digests: lanes 1, 5, 9, 13, without any enzyme digestion; lanes 2, 6, 10, 14, digested with *PvuII*; lanes 3, 7, 11, 15, digested with *BspO4I*; lanes 4, 8, 12, 16, digested with *PvuII* and *BspO4I*; lane 17, a molecular weight marker pHY.

Figure 2. Determination of cleavage site. Lanes F and R are products made with the forward and reverse primers, respectively.

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