A restriction enzyme, *BpuI* is an isoschizomer of *BanII*

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We previously identified a type II restriction enzyme in extract of *Bacillus pumilus* AHU1387 and designated it *BpuI* (1, 2), which recognizes the sequence GRGCTC and therefore may be an isoschizomer of *HgiIII* or *BanII* (3). In the present study, we examined the cleavage site of *BpuI* as well as the effect of methylation on the cleavage reaction in order to show that *BpuI* is an isoschizomer of *BanII*.

B. pumilus AHU1387 was cultured at 37°C for 20 hours in a nutrient broth and the collected cells were disrupted by treatment with lysozyme and sonication. The restriction enzyme was purified from the cell-free extracts by Polymin P fractionation, ammonium sulfate precipitation and the column chromatographies on phosphocellulose, Heparin-cellulofine and double stranded DNA-cellulose. The purified enzyme required 2–10 mM MgCl₂ and 100–200 mM NaCl (or 50–200 mM KCl) for maximum expression of the activity. It acted over a wide range of pH (7.5–9) and temperature (37–50°C).

BpuI cleaved λ , pUC19, pBR322, adenovirus type2 DNAs at 7, 1, 2, and more than ten sites, respectively. The cleavage patterns were identical with those obtained by *BanII* (Figure 1). A double digestion with *BpuI* and *BanII* suggested that these two enzymes cleave at the same sites on DNA. The cleavage site of *BpuI* was determined by primer extension reaction. Plasmid pUC19 DNA, which has a unique site for *BpuI*, was cleaved with *BpuI* and then annealed with appropriate primers. The primers were extended with Klenow fragment in the presence of [α -³²P]dCTP. The extension reactions were performed with both directions. Dideoxy sequencing reaction with the same primers was also carried out and electrophoresed in parallel with the primer-extended products. As shown in Figure 2, the primer extension reactions were terminated at the first G residue in the sequence GAGCTC. This result shows that *BpuI* cleaved the phosphodiester bond between T and C in the above sequence.

The effect of methylation on the cleavage reaction by *BpuI* was examined in the following manner. Plasmid pUC19 and phage λ DNAs were used as substrate for methylation and digestion. The substrate DNAs were methylated by *AluI* methylase, which converted the cytosine in the sequence AGCT to 5-methylcytosine. The methylated DNAs were then cut by either *BpuI* or *BanII*. The cleavage patterns by both enzymes were the same showing that *BpuI*, like *BanII*, does not cleave the sequence GAG^mCTC. From these data we concluded that *BpuI* is an isoschizomer of *BanII*.

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Figure 1. The cleavage patterns of *BpuI* on various DNAs. A, λ ; B, pUC19; C, pBR322 and D, adenovirus type 2 DNA. Lanes 1, 4, 7 and 10, untreated; lanes 2, 5, 8 and 11, *BanII*-treated; lanes 3, 6, 9 and 12, *BpuI*-treated.

Figure 2. Determination of cleavage site. The triangle indicates the termination point of the primer extension reaction. Lanes F and R indicate the primer-extended products with the forward and reverse primer, respectively.