Isolation and identification of the restriction endonuclease PtaI from *Phormidium tadzschicum*, an isoschizomer of BspMII

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Received June 2, 1992; Revised and Accepted October 27, 1992

PtaI is a type II restriction endonuclease from the Cyanobacterial strain *Phormidium tadzschicum*. This strain was obtained from the Collection of Algal Cultures of Leningrad University. PtaI recognizes the sequence 5'-TCCGGA-3' and cleaves between T and C. The enzyme was purified using the following chromatographic steps: 1) Phosphocelulose, 2) DEAE-Sephadex. The enzyme was free of contaminating nucleases. Optimal conditions for PtaI digestion are 20 mM Tris (pH 7.9), 10 mM MgCl₂, 50 mM KCl, 100 µg/ml BSA, 1 mM DTT and incubation at 37°C. PtaI cleaves lambda DNA giving the same digestion pattern as BspMII. DNA sequencing (Figure 2) confirmed that PtaI is an isoschizomer of BspMII.

Figure 1. To verify that PtaI and BspMII share a recognition sequence, lambda DNA was digested with: lane 1) BspMII, lane 2) PtaI, lane 3) BspMII + PtaI, lane 4) lambda DNA undigested.

ACKNOWLEDGMENTS

This work was supported by Polish American Joint Fund II Grant No: MEN/NSF-89-9.

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Figure 2. The cleavage site of PtaI was determined as follow: pBR322 dsDNA was denatured to single strand and then annealed with a 20 mer synthetic oligonucleotide of sequence 5'-AACATGAATGGTCTTCGGTT-3' at position 1591. Using TaqTrack sequencing system the primer was extended through the PtaI site at position 1664 and digested with PtaI. The cleavage product (lane 1) comigrates with the band corresponding to the T in the sequence 3'-AGGCC-T-5', therefore PtaI recognizes and cleaves the following sequence 5'-T/CCGGA-3'.