BmyI, a novel *SduI* isoschizomer from *Bacillus mycoides* recognizing 5'-GDGCH/C-3'

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We have isolated *BmyI*, a novel class-II (1) restriction endonuclease from *Bacillus mycoides* recognizing the palindromic sequence 5'-GDGCH/C-3' generating 3'-protruding DGCH-tetranucleotides. With respect to its isoschizomers *AocII*, *Bsp1286I*, *NspII* or *SduI*, the novel enzyme can be isolated in high purity because of its occurrence as a single restriction enzyme in the *Bacillus mycoides* strain and presence of high specific activity even in the crude extract.

A comparison of cleavage patterns experimentally obtained with *BmyI* on standard lambda, Ad-2, SV40, ϕ X174, M13mp7, pBR322 and pBR328 DNAs of known nucleotide sequence (Fig. 1, lanes 2–8) with computer-derived mapping data (2) predicts the sequence 5'-GDGCHC-3'. The recognition sequence was confirmed by parallel digestion of M13mp7 DNA with its isoschizomer *Bsp1286I* (3) (Fig. 1, lane 9) resulting in both cases in fragments of approximately 3700, 2700, 700 and 200 bp, which correlated with the computer-derived fragment lengths of 3647, 2655, 722 and 178 bp for the sequence 5'-GDGCHC-3'.

The cut positions within the *BmyI* recognition site were determined according to the enzymatic sequencing approach described in (4). An M13mp18-derivative with an insert containing an *BmyI* cleavage site was used for enzymatic sequencing reactions starting with a 5'-phosphorylated universal M13 sequencing primer. In a parallel reaction, the same primer, [³²P]-end-labeled with T4 PNK and [γ -³²P]ATP, was annealed to the template, and the labeled primer was extended by treatment with Klenow enzyme and all four dNTPs through the *BmyI* site. The double-stranded DNA was used as substrate for *BmyI* to produce 5'-end-labeled DNA fragments comparable to the sequencing ladder. Samples were analyzed without or with (–/+) further incubation with T4 DNAP and all four dNTPs by electrophoresis and subsequent autoradiography (Fig. 2). In the *BmyI* reaction the observed single band comigrated with T(5); after T4 DNAP treatment, the observed band shifts to G(1) of the recognition sequence 5'-GAGCTC-3'.

From the mapping and sequencing data the specificity of *BmyI* is concluded as:

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Figure 1. *BmyI* digests on lambda (2), Ad-2 (3), SV40 (4), ϕ X174 (5), M13mp7 (6), pBR322 (7), pBR328 (8). (9): M13mp7[*SduI*]-fragments. (1, 10): MW marker.

Figure 2. Determination of *BmyI* cleavage positions.