Isolation and identification of restriction endonuclease *Aor51HI* from *Acidiphilium organovorum* 51H

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Aor51HI, a type II restriction endonuclease, has been isolated from *Acidiphilium organovorum* 51H. *Aor51HI*, an isoschizomer of *Eco47III* (1), recognizes the palindromic six base sequence 5'-AGCGCT-3', and cleaves the center of recognition sequence giving the blunted end.

The enzyme was purified using the following chromatographic steps: 1) phosphocellulose, 2) DEAE-cellulose, 3) heparin-Sepharose. The prepared enzyme was free of contaminating nuclease and phosphatase activities. The crude extract contained approximately 50,000 units *Aor51HI* per gram of wet cells. Optimum conditions for *Aor51HI* activity are 10 mM Tris-HCl (pH 8.0), 7 mM MgCl₂, 7 mM β-mercaptoethanol, 60 mM KCl at 37°C. The fragments produced by *Aor51HI* digestion of Lambda, Adenovirus-2, SV40 and pBR322 DNAs match those predicted by cleavage at the sequence AGCGCT (Figure 1, lanes 1-5).

The cleavage site of *Aor51HI* was determined by cleavage of a primed synthesis reaction (2). An M13mp18-derivative with an insert containing an *Aor51HI* cleavage site was used for enzymatic sequencing reactions starting with a 5'-[³²P] phosphorylated universal M13 sequencing primer. The four standard dideoxy DNA sequencing reactions (Figure 2, lanes G, A, T, and C) and a fifth reaction containing no dideoxy terminators were extended through the *Aor51HI* site. The double-stranded DNA recovered from the fifth reaction was used as the substrate for *Aor51HI*. The cleaved product resulted in a single band (Figure 2, lane -) which comigrates with the first C in the sequence 5'-AGCGCT-3'. After the blunting reaction with T4 DNA polymerase, the migration point of cleaved product was not changed (Figure 2, lane +). These results indicate that *Aor51HI* recognizes and cleaves the following sequence:

5'-AGC↓GCT-3'
3'-TCG↑CGA-5'

Figure 1. *Aor51HI* digests of DNA: Lane 1, Lambda; 2, Adenovirus-2; 3, SV40; 4, pBR322. Marker: lane 5, λ- *Hind*III digest.

Figure 2. Determination of *Aor51HI* cleavage site.

REFERENCES

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