*Esp*3I — a novel type IIs restriction endonuclease from *Hafnia alvei* that recognizes the sequence 5′-CGTCTC(N)$_{1/5}$-3′

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A new type IIs restriction endonuclease, *Esp*3I, has been isolated from *Hafnia alvei* RFL3*. *Esp*3I recognizes the non-palindromic sequence 5′-CGTCTC-3′ and cleaves DNA one nucleotide to the right from the noted recognition sequence and five nucleotides to the right on the opposite strand.

The *Esp*3I recognition sequence was determined by mapping the restriction sites on plasmid pBR322 and lambda DNAs. The single cleavage site of *Esp*3I on pBR322 DNA was mapped to an approximate position 2114 with *Hin*dIII, *Eco*31I and *Eco*130I (data not shown). *Esp*3I cleaves lambda DNA approximately at 13 sites, five of them (16700, 19400, 20460, 37260, 47330) were mapped with *Kpn*I, *Nae*I, *Xba*I, *Ehe*I, *Xho*I, *Eco*31I and *Eco*81I (not shown). Search of homologous nucleotide sequences on computer let us find the only common sequence, (5′-CGTCT-C-3′), for all the positions mapped. The predictive cleavage patterns on phages lambda, phiX174 and plasmid pBR322 DNAs, at the sequence 5′-CGTCTC, match the experimentally observed *Esp*3I digests of these substrates, from which we conclude that *Esp*3I recognizes the sequence above.

The cleavage site of *Esp*3I was determined using synthetic oligonucleotide duplex, which contains the *Esp*3I recognition sequence (boxed):

5′-GG CGTCTC GCGATACC-3′ [I]
3′-CC GCAGAG CGCTATGG-5′ [II]

Each of the oligonucleotides were ^{32}P-labeled and annealed with complementary unlabeled oligonucleotide. The duplexes obtained were cleaved with *Esp*3I and the cleavage products were analyzed by homochromatography on DEAE−cellulose thin-layer plate using homomixture VI (2). Partial snake venom phosphodiesterase digests of the same ^{32}P-labeled oligonucleotides were used as authentic markers. Autoradiograms are demonstrated in Figure 1. Obtained ^{32}P-labeled nonanucleotide (lane 2) and trinucleotide (lane 3) indicate that *Esp*3I cleaves oligonucleotide duplex outside the recognition site producing a four base 5′-extension, as below:

5′-CGTCTCN↓
3′-GCAGAGNNNNN↑

*Esp*3I — is a new type IIs prototype recognizing non-palindromic hexanucleotide sequence. No isoschizomeric restriction endonuclease recognizing the same sequence has been previously described (1). The *Esp*3I recognition site possess a pentanucleotide 5′-GTCTC which occurs in the sequence recognized by *Eco*31I (5′-GGTCTC) (3). This pentanucleotide also is another type IIs restriction endonuclease *Bsm*AI recognition sequence (4).

REFERENCES

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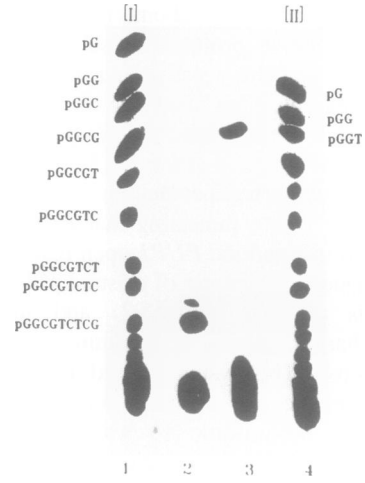

Figure 1. Determination of the cleavage site of *Esp*3I using synthetic oligonucleotide duplexes. Lanes 1 and 4: partial digestion products of top [I] or bottom [II] oligonucleotides; lanes 2 and 4: *Esp*3I digests of duplexes containing ^{32}P-labeled top [I] or bottom [II] oligonucleotides.

*The bacterial strain was primarily identified as *Erwinia species* RFL3 whereas R−M enzymes discovered were designated *Esp*3I. Closer examination of the taxonomy of microorganism caused its reidentification as *Hafnia alvei*. The name of R−M enzymes, however, following the practice used by Dr R.Roberts in regularly prepared compilations of the restriction enzymes (1), was not changed.