Isolation and identification of two new *Synechococcus*-derived restriction endonucleases, *SleI* and *SspAI*, isoschisomers of *EcoRII*

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Two new type-II restriction endonucleases, *SleI* and *SspAI*, have been isolated from *Synechococcus leopoliensis*. Strain 1402-1 was obtained from the Institut Pasteur Culture Collection of Cyanobacterial Strains. *Synechococcus* sp. AN6301 was obtained from Pflanzenphysiologisches Institut, Universität Göttingen, Nikolausberg, FRG.

Both strains were grown aerobically at 30°C in BG medium (1) under fluorescent light. The cells were harvested and stored in liquid nitrogen. Frozen cells were disrupted by sonication. Purification of the enzymes was carried out by the following steps: (I) DEAE-cellulose chromatography, (II) DNA-cellulose chromatography, (III) QAE-Sephadex chromatography.

The digestion pattern of pBR322 DNA with *BstNI* was identical to the patterns obtained with *SleI* and *SspAI*, indicating that *SleI* and *SspAI* are isoschisomers of *BstNI*, and therefore, of its isoschisomer *EcoRII* (Fig. 1).

It was found that *SleI* and *SspAI* enzymes generate 5 nucleotide (nt) cohesive ends, as assessed by digestion of lambda DNA and fill-in reaction with T7 DNA polymerase, [α -³⁵S]dATP and the other dNTPs. *EcoRII* and *BstNI* recognize the same sequence, but cut between different nt generating 5-nt and 1-nt cohesive ends, respectively. Generally following the approach described by Brown *et al.* (2), we have directly determined the sequence of the cleavage site of *SleI* and *SspAI* in M13mp18 double stranded DNA. The first restriction site recognized by *EcoRII* is located 167 nt from the first nt of the 17-mer sequencing primer, and thus can easily be sequenced. We found that the *SleI* and *SspAI* cut sites (represented by arrows) are shifted by two nt (Fig. 2) within the recognition site with respect to the *BstNI* cuts (represented by dots), and thus are identical to the *EcoRII* cuts.

Figure 1. pBR322 DNA digested with: *BstNI* (lane 2), *EcoRII* (lane 3), *EcoRII* + *BamHI* (lane 4), *SleI* (lane 5), *SleI* + *BamHI* (lane 6), *SspAI* (lane 7), *SspAI* + *BamHI* (lane 8); lane 1 represents size standards.

Figure 2. Determination of the recognition sequence and cleavage sites of the following enzymes on M13mp18 DNA: *SleI* (lane 1), *SspAI* (lane 2), *BstNI* (lane 3).

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