รูปแบบ Abtract (บทคัดย่อ)

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Project title: การจำแนกและการทำบริสุทธิ์ของ Restriction Endonucleases จาก *Streptomyces* (Identification and Purification of Novel Restriction Endonuclease from Two Hundred Species of Unidentified *Stretpomyces*

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Abstract (สรุปย่อ)

Forty strains of unidentified *Streptomyces* isolated from soil samples collected in a deciduous dipterocarpe forest in Thailand were screened for novel restriction endonuclease. Four of unidentified strains of *Streptomyces*, designated as DC1, DC12, DC13 and DC81, were detected to contain specific endonucleases. In search of novel restriction endonuclease, specific endonucleases from *Streptomyces* DC12 and DC13 were partially purified and characterized.

Two different restriction endonucleases had been purified from 200 grams of *Streptomyces* DC12. Purification of these endonucleases involved removal of nucleic acids by addition of streptomycin sulfate, ammonium sulfate fractionation, DEAE-cellulose and CM-cellulose chromatography. With DEAE-cellulose chromatography, the enzymes resolved into two active fractions, designated as *Sdc*AI and *Sdc*AII, respectively. The specific activities of the purified *Sdc*AI and *Sdc*AII from *Streptomyces* DC12 were 50 and 190 units/mg, respectively. Similarly, two different restriction endonucleases had been purified from *Streptomyces* DC13. When loaded onto DEAE-cellulose, the restriction endonucleases also resolved into two active fractions, designated as *Sdc*BI and *Sdc*BII, respectively. The specific activities of the purified *Sdc*AI and *Sdc*AII from *Streptomyces* DC13 were 267 and 228 units/mg, respectively. Analysis of these purified proteins on SDS-PAGE showed that the enzymes still contaminated with other proteins.

Substrate specificity and kinetic cleavage of plasmid by *SdA*I, *Sdc*AII, *Sdc*BI, and *Sdc*BII were characterized. All these enzymes required Mg^{2+} for catalytic activity with the optimum temperature at 37°C and cleaved super-coiled pBluescript, pUC118, pET-15b, and pET-26b only once. However, unlike type II restriction enzyme *Eco*RI, cleavage of these super-coiled plasmids resulted in formation of mostly circular product rather than linear product. When linear pBluescript was used as substrate for *Sdc*AI and *Sdc*BI, no cleavage activity was observed. This result suggested two possibilities. First, the cleavage sites of *Sdc*AI and *Sdc*BI are located in close proximity to the cleavage site of *Eco*RI. Second, *Sdc*AI and *Sdc*BI nicked the linear pBluescript that cannot be resolved from the non-nicked linear pBluescript on agarose gel. A computer-aided search of homologous nucleotide sequences of all four plasmids revealed at least four possible nucleotide sequences with 6 bp in length, which located either inside or within the origin of replication of the plasmid. PCR generated 500 bp, 1000 bp, and 1500 bp fragments that contained these possible sequences were used as substrates for *Sdc*AI and *Sdc*BI. The result suggested 500 bp, 1000 bp, and 1,500 bp PCR fragments were nicked instead of cleaved on both strand by these enzymes. The exact cleavage sites of *Sdc*AI, *Sdc*AII, *Sdc*BI, and *Sdc*BII are remained to be determined.

Kinetic cleavage of pBluescript by *Sdc*AI, *Sdc*AII, *Sdc*BI, and *Sdc*BII was compared to that of *Eco*RI, a type II restriction endonuclease. With *Eco*RI, cleavage of super-coiled pBluescript generated mostly linear product, with transient accumulation of circular product at early state. Such cleavage pattern is consistent with a concerted mechanism. With *Sdc*AI, *Sdc*AII, *Sdc*BI, and *Sdc*BII, cleavage of super-coiled pBluescript produced mostly circular product, with minor linear product. This data indicated that these enzymes rapidly nicked one strand of super-coiled DNA and converted it into the open circular form, which was subsequently converted to linear form at slower rate. Such DNA cleavage pattern is consistent with a step-wise mechanism that had been proposed for the type IIs nicking restriction endonuclease, N.*Bst*NBI. It appears proposed that *Sdc*AI, *Sdc*AII, *Sdc*BI, and *Sdc*BII are novel type IIs nicking restriction endonucleases that had never been isolated before until this study. It is proposed that the nicking endonuclease purified in this study also existed in solution as monomer and bind to their recognition site as monomer. Unlike typical type II restriction endonuclease such as *Eco*RI, the nicking endonucleases purified in this study cannot dimierize due to mutation in their dimerization domains. Thus, cleavage of the super-coiled plasmid resulted in the formation of mostly circular product rather than linear product.

Key words: Type IIs restriction endonuclease, nicking endonuclease, *Streptomyces*, DNA cleavage mechanism.