Restriction endonuclease from thermophilic bacterial species III. Isolation and characterization of \textit{BsiHKA} I

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\textit{BsiHKA} I is a type II restriction endonuclease from a \textit{Bacillus stearothermophilus} strain isolated from soil according to (1). \textit{BsiHKA} I in 1 l culture (4.7 g wet cells) was purified by a DEAE-sephacel column (30 ml bed volume). Enzyme eluted at about 0.3 M NaCl was dialysed against buffer A (1) and loaded onto a heparin column (8 ml bed volume). Enzyme eluted at 0.4–0.5 M NaCl was dialysed against buffer B (1) and loaded onto an FPLC Mono Q anion exchange column. Enzyme was eluted at 0.3–0.4 M NaCl.

The purified enzyme was used to digest various DNA with known sequences (Fig. 1). The sizes and numbers of fragments produced are identical to those cleaved by mesophilic enzyme \textit{HgiAl} which recognizes 5'G(AT)GC(AT)C3' (2).

The cleavage site of \textit{BsiHKA} I was determined according to (3) using pUC18 DNA as the template and a 17 mer oligonucleotide with sequence 5'CAGCACTGACCCGCTTTC3' as the primer. The primer was annealed to position 359–375 of the denatured pUC18 DNA and was extended through the \textit{BsiHKA} I site at position 444. Figure 2 shows that the cleavage product of reaction I comigrates with the band corresponding to nucleotide T in the sequence GAGCTC and reaction II comigrates with the band corresponding to the first G nucleotide. Therefore, \textit{BsiHKA} I recognizes and cleaves 5'G(AT)GC(AT)C3'.

The optimal buffer for this enzyme was medium salt (4) and optimal reaction temperature 60°C.

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REFERENCES


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