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AccBSI: A New Restriction Endonuclease from *Acinetobacter calcoaceticus* BS

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The recognition site of a new restriction endonuclease from Acinetobacter calcoaceticus BS was determined. This is a nonpalindromic sequence

5'-GAGCGG-3'
3'-CTCGCC-5'

AccBSI restriction endonuclease cleaves DNA chains in the middle of the recognition sequence; therefore, ligation of its digestion fragments restores AccBSI recognition sites and generates palindromic sequences recognized by SacI and SacII restriction endonucleases.

In spite of a considerable amount of annually discovered bacterial strains producing class II restriction endonucleases [1], only few of them are used for commercial production. The remaining strains are insufficiently productive, or there are isoschizomers with the same properties.

We isolated a new restriction endonuclease AccBSI, which is of interest for both fundamental research and practical purposes because of its unusual recognition sequence and the abundance of this enzyme in cells of the producer strain.

We characterized the specificity of this endonuclease and determined the phosphodiester bonds to be cleaved.

MATERIALS AND METHODS

The bacterial strain BS was isolated from a natural fresh water sample. We identified it as *Acinetobacter calcoaceticus* according to [2] and named it following the conventional nomenclature [3].

The strain was grown in a shaker bath at 120 rpm and 30°C in the following broth: 7 g/l peptone, 3 g/l

yeast extract, 1 g/1 potassium acetate, and 1 g/1 NaCl. After 16 h of incubation, the cells were harvested by centrifugation and sonicated. The enzyme was isolated by sequential steps of chromatographic purification on P-II phosphocellulose, DEAE cellulose and heparinSepharose. The preparation was tested for the absence of nonspecific nucleases and phosphatases in experiments with a radio-labeled oligonucleotide substrate as described in [4].

Digestion of DNA by endonuclease AccBSI was performed at 37°C in a buffer containing 30 mM Tris acetate, 10 mM magnesium acetate, 70 mM potassium acetate, and 1 mM dithiothreitol, which was optimal for the enzyme. The resulting digestion products were applied to a 1 % agarose gel, fractionated by electrophoresis in Tris-borate buffer with ethidium bromide, and photographed under ultraviolet illumination.

A modified Maxam-Gilbert procedure [5] was used for the cleavage sites determination.

RESULTS AND DISCUSSION

To determine the sequence recognized by the enzyme, several types of viral and plasmid DNA

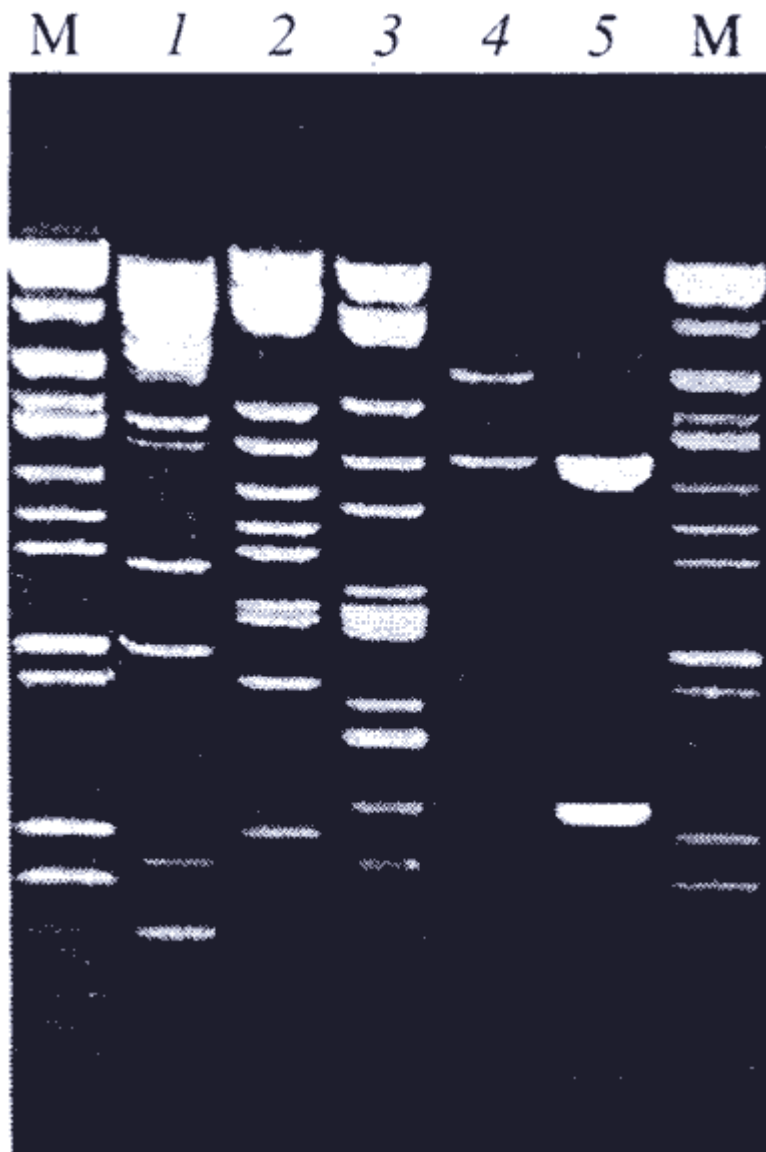
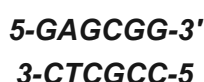


Fig. 1. Digestion of various DNA with AccBSI restriction endonuclease.

(1) Lambda phage; (2) T7; (3) adenovirus 2; (4) pBR322, (5) pUC19; M, DNA fragment length marker (lambda DNA digested with Bme 18I).

were completely digested with AccBSI. The resulting electrophoretic patterns (Fig. 1) were compared with computer-simulated patterns of digestion of corresponding DNAs with more than 200 currently known restriction enzymes [1]. The pattern produced by the enzyme was found to be similar to that of BsrBI which recognizes the nonpalindromic nucleotide sequence



To define the sites of DNA cleavage by the new enzyme, two α - ^{32}P -labeled fragments of the plasmid pUC19 EcoRI-PvuII (100 bp) and CfrI-PstI (206 bp), were sequenced. The restriction endonuclease AccBSI was found to cut both strands of DNA in the middle of the recognition sequence (Fig. 2), as indicated by arrows:



Hence, the restriction endonuclease AccBSI is a true isoschizomer of BsrBI isolated from *Bacillus stearothermophilus* [6]. Further studies showed that the enzyme, unlike its analog, is heat sensitive and inactivates completely after 20 min of incubation at 65°C.

The blunt ends formation after DNA cleavage is not typical for restriction endonucleases with nonpalindromic recognition sequences. In addition to BsrBI and AccBSI, only one such restriction endonuclease Mlvi, which is not available commercially, cuts DNA in a similar manner; however, its cleavage site is outside the recognition sequence. A symmetrical cleavage within a nonpalindromic recognition site (but not blunt end producing) is not typical either. Few such restriction endonucleases are known: BsiI BpuIOI [7], and Acil [8].

In addition to a restoration of the original recognition sites, ligation of AccBSI digestion fragments results in six-letter palindromic sequences recognized by restriction endonucleases SacI (GAGCT $^{\wedge}$ C) and SacII (CCGC $^{\wedge}$ GG). These sequences are formed by halves of the corresponding recognition sites separated by the cleaved bond.

In several cases, isoschizomers of these restriction enzymes are produced by the same strains (SacI and SacII, SstI and SstII as well as Uba 1094I and Uba1094II). This suggests that the gene for restrictase AccBSI may have resulted from fusion of genes for two endonucleases presented in one bacterial cell but differing in site-specificity, which combine domains involved in binding with the recognition sequence in one protein molecule.

Formation of recognition sites for other enzymes during ligation of AccBSI digests may provide an advantage for gene engineering studies.

We emphasize that the conventional test for the absence of exonuclease impurities in preparations of site-specific endonucleases, which includes sequential digestion-ligation-digestion [6] (Fig. 3), cannot be applied to the enzyme considered, since about 50% of recognition sites are lost after ligation. The necessary sensitivity can be achieved by using an oligonucleotide substrate instead [4].

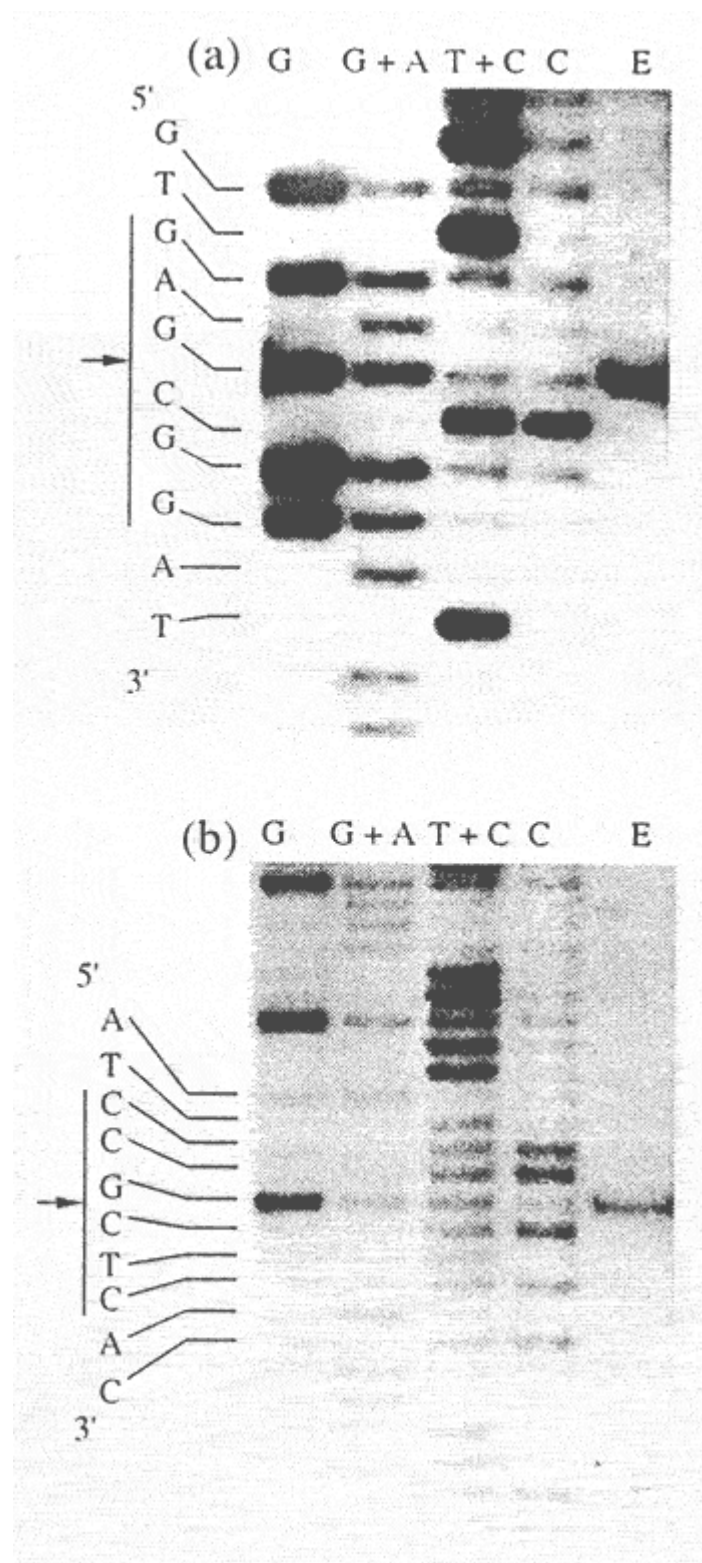


Fig. 2. Determination of cleavage sites of AccBSI restriction endonuclease.

Sequencing of α - ^{32}P labeled (a) EcoRI-PvuII and (b) CfrI-PstI fragments of pUC19 by a modified Maxam-Gilbert method. E-products of enzymatic digestion of DNA fragments with AccBSI endonuclease.

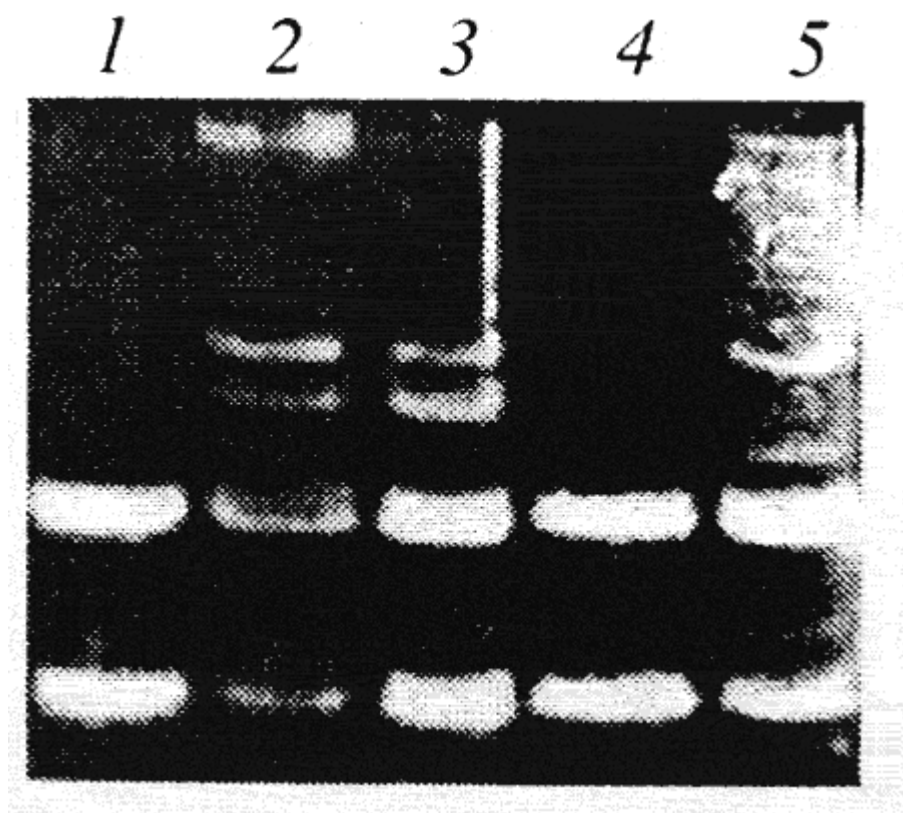


Fig. 3. Ligation of pBR322 digested with AccBSI and treatment of the ligation products with restrictases AccBSI, Psp124BI (isoschizomer of SacI), and Sfr303I (isoschizomer SacII).

(1) pBR322 digested with AccBSI; (2) ligation of the digest shown in lane 1; (3) redigestion of the ligation products with AccBSI; (4) double digestion of the ligation products with Psp124BI, (5) Sfr303I, and AccBSI; double digestion of the ligation products with Psp124BI and Sfr303I; M, DNA fragment length marker (lambda DNA digested with BmeI8I).

AccBSI is effectively produced by *A. calcoaceticus* BS. The enzymatic activity of the cell mass is approximately 300 000 U/g, which exceeds the usual level for subclass II-S restrictases by two or three orders of magnitude. The purity and high activity of AccBSI preparations allow their successful use in molecular biology experiments.

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