BepI restriction endonuclease, a new isoschisomer of FnuDII

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A new TypeII restriction endonuclease was isolated and characterized from a bacterial strain identified as Brevibacterium epidermidis by Dr.M.Konkoly-Thege (Budapest). The strain grows well in yeast-trypton broth aerobically with a doubling time of 50 min at 25-30°C. The enzyme was purified by a conventional procedure, employing Bio-Gel A-0.5 m, DEAE-cellulose and phosphocellulose chromatography. The enzyme at this stage of purification was applicable for all practical purposes, it was virtually free from contaminating nucleases. The yield of the enzyme was approximately 3-400 units/g wet weight of cell.

The optimal conditions for the activity of the enzyme are as follows: temperature 25-30°C, Mg²⁺ ions 10 mM, pH 7.4, no salts.

The cleavage pattern of the enzyme tested with known DNA molecules (lambda phage, pBR322) was identical with the pattern obtained with FnuDII. This identity was confirmed by parallel digestions with the new enzyme and commercial FnuDII, and double digestions with both enzymes, that resulted in unaltered digestion patterns. The cleavage site was established by running a Maxam-Gilbert sequence gel from a DNA fragment of known sequence, which contained a FnuDII site. The same end-labelled DNA fragment was then digested with the new enzyme, and it was electrophoresed alongside the sequence ladder. This experiment unambiguously proved that the cleavage occurred in the middle of the palindromic CGCG sequence, generating flush ends.

Thus the enzyme does not represent a new specificity, it is an isoschisomer of the well known FnuDII enzyme (1). Nevertheless it offers some practical advantages. The Fusobacterium nucleatum strain, from which FnuDII is isolated, is an obligately anaerobic pathogenic microorganism, therefore FnuDII is fairly expensive. The only other commercially available isoschisomer, ThaI is purified from an extremely thermophilic and acidophilic organism (2), the other described isoschisomers (3, 4) have not been characterized. It is hoped that the BepI enzyme will be a better substitute for these isoschisomers.

References

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