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Two restriction endonucleases from *Bacillus sphaericus*: BspXI and BspXII

M.Zieger, M.Patillon[1], G.Roizes[2], T.Lerouge[3], D.Dupret* and J.M.Jeltsch[3]

Appligène, BP 72, 67402 Illkirch Cédex, [1]Cytopathologie Végétale et Toxicologie Cellulaire, Université de Paris VII, 2 Place Jussieu, Paris Cédex 05, [2]INSERM U.249 et LP 8402 du CNRS, Boulevard Henri IV, 34060 Montpellier Cédex and [3]INSERM U.184 et LGME du CNRS, 11 rue Humann, 67085 Strasbourg Cédex, France
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In screening wild strains of Bacillus sphaericus, one, which we call X -not totally identifiable when compared to well established strains- exhibited a high yield of two restriction endonucleases : BspXI and BspXII. A cleared sonic extract, obtained from 12 grams of frozen cells yielded as much as 500,000 units of each enzyme by the following steps of chromatography and dialysis [against PC buffer (10% glycerol, 10 mM KPO_4-pH 7.4, 10 mM β-mercaptoethanol, 0.1 mM EDTA)]. (I) A Biogel A-0.5 m filtration (II) Dialysis of active fractions (III) DEAE Sephacel chromatography : a gradient elution from 0 to 1 M NaCl gave pool I (0-0.5 M NaCl) and pool II (0.5-1 M NaCl). (IV-VI) Dialysed pool I was loaded on phosphocellulose column and eluted with a 0 to 1 M KCl gradient; the fraction at 0.55 M KCl was dialysed and the phosphocellulose chromatographic step repeated, yielding, at 0.55 M KCl, a pure BspXI fraction. (VII) Dialysed pool II was applied on a Blue Trisacryl column and eluted with a 0 to 0.5 M KCl gradient giving, at 0.3 M KCl, a pure BspXII fraction. The digestion patterns of different DNAs (λ, pBR322 and Ad2) with BspXI and BspXII were identical to those obtained with ClaI and BclI, respectively, or with BspXI + ClaI and BspXII + BclI. It was therefore concluded that BspXI and BspXII are isoschizomers of ClaI (5'-AT/CGAT-3') and BclI (5'-T/GATCA-3') respectively (1, 2).

BspXI was further investigated using : [a] standard procedures (3) to determine the cleavage site at the nucleotide level and [b] a recombinant DNA (4) that contains a ClaI recognition sequence cleaved by the enzyme only when it is produced in an E.coli dam$^-$ strain. Since BspXI behaves exactly like ClaI (sensitive to N^6-adenine methylation - generates 5' protruding dinucleotide CG), BspXI is a full isoschizomer of ClaI with the property that this new enzyme is able to cleave DNA at 37°C in standard buffers containing from 0 to 200 mM NaCl without any noticeable loss of activity.

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* To whom correspondence should be addressed.