A RAPID PROCEDURE FOR THE ISOLATION OF ENDONUCLEASES FROM TWO THERMOPHILIC BACTERIA

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1. Introduction

A method for the partial purification of the endonuclease *Taq*I from the extreme thermophile *Thermus aquaticus* has been reported in [1]. We describe here a rapid and easy procedure suitable for the isolation of *Taq*I and *Bst*I, an endonuclease with *Bam*-like specificity [2] from the moderate thermophile *Bacillus stearothermophilus*.

2. Materials and methods

Thermus aquaticus (ATCC 25104) and *Bacillus stearothermophilus* (strain NCA 1503) cells were obtained from the Microbiological Research Establishment, Porton Down, Wiltshire, and stored at −20°C. Cells (100 g) were thawed in 100 ml buffer A (20 mM potassium phosphate (pH 6.8), 1 mM 2-mercaptoethanol) at 4°C and disrupted by means of the French pressure cell at 110–140 kg/cm^2. The *B. stearothermophilus* cell extract was centrifuged at 40 000 × *g* for 60 min at 4°C. The *T. aquaticus* cell extract contains a pigmented 'slime' which interferes with later purification steps and so was centrifuged at 100 000 × *g* for 120 min at 4°C to remove this. Streptomycin sulphate (5 g) was added with stirring to the clear supernatant as a 10% solution in buffer A over a period of 30 min at 4°C. The resulting precipitate was removed by centrifugation at 40 000 × *g* for 60 min at 4°C. The supernatant was dialysed for 16 h at 4°C against 10 l buffer A and stirred for 30 min with a suspension of phosphocellulose (50 g Whatman P-11 in buffer A) which was collected and washed with 3 vol. buffer A (200 ml each) on a sintered glass funnel. The phosphocellulose was suspended in buffer A and poured into a glass column (30 × 2 cm) and developed with a linear gradient (800 ml) of KCl from 0–0.8 M. Endonuclease active fractions (5 ml) were pooled and dialysed for 24 h at 4°C against buffer A containing 50% glycerol and stored at −20°C.

*Taq*I and *Bst*I activity was determined by incubating 2 μl samples at 60°C for 1 h in a reaction mixture (15 μl) containing 1 μg bacteriophage λ DNA, 12 mM Tris–HCl (pH 7.4), 12 mM $MgCl_2$, 12 mM 2-mercaptoethanol. The digestion was stopped by adding 5 μl of a solution containing 50% sucrose, 0.1 M EDTA, 0.08% bromophenol blue and applied onto a 5% polyacrylamide slab gel run at 150 V for 2 h.

3. Results and discussion

Active fractions of both *Taq*I and *Bst*I from phosphocellulose gave distinct and characteristic gel electrophoresis patterns with DNA. *Taq*I eluted from phosphocellulose at 0.30–0.45 M KCl (fractions 60–90, fig.1(a)) and *Bst*I at 0.20–0.39 M KCl (fractions 40–76, fig.1(b)). *Bst*I has the same specificity as *Bam*I [2] recognising and cleaving the hexanucleotide sequence:

G–G–A–T–C–C

It has the advantage over *Bam*I of being stable and

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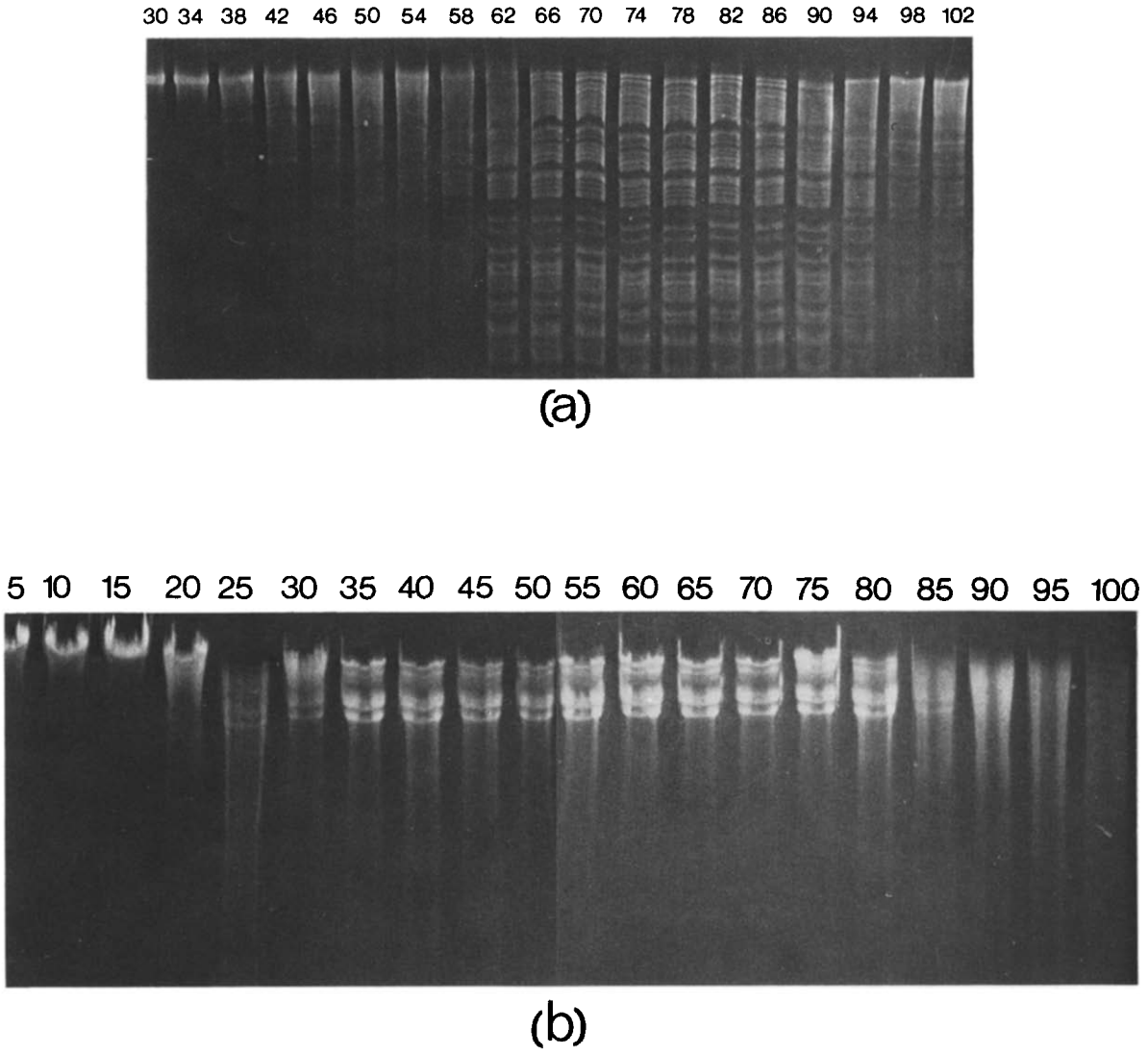

Fig.1. Gel electrophoresis patterns showing endonuclease activity from (a) *T. aquaticus* and (b) *B. stearothermophilus* from the phosphocellulose column. Numbers above refer to fraction numbers.

active at 60°C and thus may prove more useful in studying DNA structure. *Taq*I recognises and cleaves the tetranucleotide sequence:

5′ T↓C–G–A 3′

3′ A–G–C↑T 5′

It shows an even higher degree of thermal stability, being completely active at 70°C, and has been valuable in the determination of the sequence of DNA from bacteriophage ϕX174 [3] and G4 (G. N. Godson, B. G. Barrell, R. Staden and J. C. Fiddes, submitted).

A second endonuclease from *T. aquaticus*, *Taq*II, has been reported and isolated [4]. *Taq*I (fractions 60–90, fig.1(a)) was isolated from other endonucleases by the procedure described here, but material which

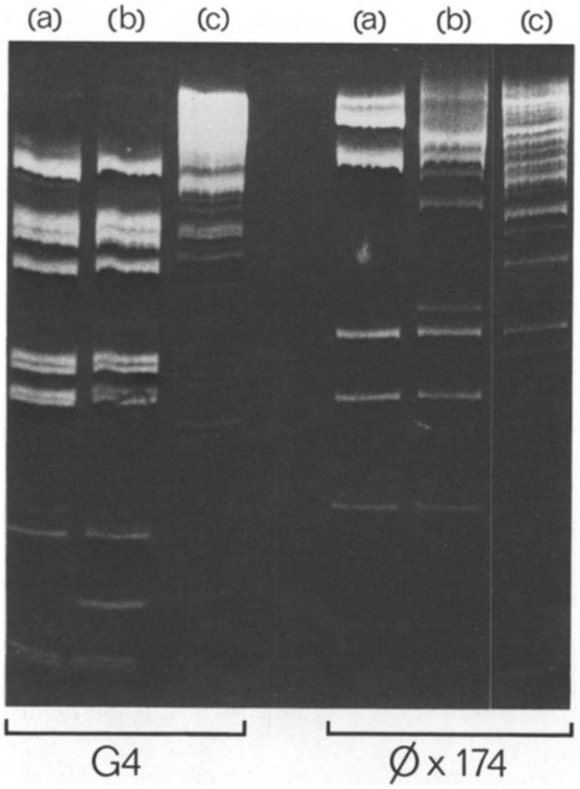

Fig.2. Gel electrophoresis patterns of (a) *Taq*I, (b) *Taq*I + *Taq*II and (c) *Taq*II digests of φX174 RF DNA and G4 RF DNA.

eluted from phosphocellulose at 0.18–0.26 M KCl contained both *Taq*I and *Taq*II activity. The gel electrophoresis pattern obtained from this material, using φX174 RF DNA and G4 RF DNA as substrate, was identical with that from a double digest using isolated *Taq*I and *Taq*II (kindly donated by T. A. Bickle). Figure 2 illustrates the minor difference in gel pattern between the *Taq*I alone and *Taq*I + *Taq*II digest. Only one new fragment is produced from G4 RF DNA and very few more from φX174 RF DNA. It is hoped that these new fragments may enable us to determine the cleavage site of *Taq*II. It is clear that this would be difficult to achieve from a *Taq*II digest alone (see fig.2).

The yield of *Taq*I (40 000 units from 100 g cells, a unit being defined as the amount required to give the limit digest in 1 h at 60°C with 1 μg DNA) is 20-times that quoted in [1] when the digest was carried out at 37°C. This yield is still much lower than for other restriction-like endonucleases from bacteria. However, the yield of *Bst*I (200 000 units from 100 g cells) is within the normal range.

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References

[1] Sato, S., Hutchison, C. A. III and Harris, J. I. (1977) Proc. Natl. Acad. Sci. USA 74, 542–546.
[2] Catterall, J. F. and Welker, N. E. (1977) J. Bacteriol. 129, 1110–1120.
[3] Sanger, F., Air, G. M., Barrell, B. G., Coulson, A. R., Fiddes, J. C., Hutchison, C. A. III, Slocombe, P. M. and Smith, M. (1977) Nature 265, 687–695.
[4] Bickle, T. A., Pirrotta, V. and Imber, R. (1977) Nucleic Acids Res. 4, 2561–2571.