A NEW RESTRICTION ENZYME FROM *ENTEROBACTER CLOACAE* (*Ecl*I)

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Received 13 May 1977

1. Introduction

Deoxyribonucleases, which recognize specific nucleotide sequences in a duplex deoxypolynucleotide chain, are designated as restriction enzymes [1]. These enzymes, which may be partially involved in the phenomenon of genetic restriction [2,3], have been isolated from many bacterial species. Among them are relatively few members of the large family of Enterobacteriaceae. We wish to report here the isolation of a restriction enzyme of type II [4] from an *Enterobacter cloacae* strain. As judged from the λ cleavage pattern obtained with this enzyme, which is designated *Ecl*I, it recognizes a sequence different from that of a large variety of other type II restriction enzymes.

2. Materials and methods

2.1. *Source of materials*

1% Agarose (Seakem) slab gels in a vertical electrophoresis apparatus (constructed in our laboratory) were run in 36 mM Tris–HCl, 30 mM NaH_2PO_4 and 10 mM EDTA, pH 7.5. The gels were stained with ethidium bromide (1 μg/ml) and photographed under ultraviolet light.

2.2. *Preparation of plasmid DNA*

The plasmid DNAs were isolated as previously described [5]. λ-DNA was prepared from λ t68c857 in *E. coli* K12W8 (obtained from H. Saedler) using standard procedures.

2.3. *Assay for restriction enzyme*

Digestion with *Ecl*I (5–10 μl/assay) was carried out in 25 mM Tris–HCl and 10–20 mM $MgCl_2$. NaCl was present at a final concentration of about 50 mM from the enzymes solution (see below). DNA, 3–5 μl (0.7 μg) in 30 mM Tris–HCl, pH 7.8, 10 mM NaCl and 5 mM EDTA were added (total vol.: 20–40 μl). The samples were incubated for 30 min at 37°C and stopped with 20 μl 20% sucrose, 2% SDS, 100 mM EDTA, 0.05% bromphenol blue.

3. Results and discussion

During the isolation of the restriction enzyme *Bam*HI [6] from a stock culture of *Bacillus amyloliquefaciens* we observed a second peak of a specific endonucleolytic activity which was different from that of *Bam*HI [6]. This activity could be attributed to a contaminating bacterium, which was present in the sample of *B. amyloliquefaciens* sent to us. This bacterium was purified and identified as *Enterobacter cloacae*.

The isolation procedure is similar to that described by Wilson and Young [6]. *E. cloacae* was grown in a

minimal salt medium (7 g/liter NH_4NO_3; 3.25 g/liter KH_2PO_4; 12.5 g/liter Na_2HPO_4; 0.1 g/liter $MgSO_4$; 1 g/liter Na-glutamate; 1 g/liter aspartic acid and 20 g/liter sucrose) to the late logarithmic or early stationary phase and harvested by centrifugation. The cells (50 g wet wt) were sonicated after suspending in buffer I (25 mM Tris–HCl, pH 7.4; 10 mM 2-mercaptoethanol) and cell debris was removed by centrifugation (all centrifugations were carried out at 20 000 rev/min, 2°C, 30 min in a Sorvall SS34 rotor). Nucleic acids were removed by streptomycin sulfate precipitation (1% final concentration). Solid ammonium sulfate was added to the supernatant with constant stirring at 4°C. The activity was precipitated between 40% and 80% $(NH_4)_2SO_4$ saturation. The precipitate was resuspended in 15 ml buffer I, applied to a bed of Sephadex G-25 (5 × 33 cm) and eluted with 10 mM potassium phosphate, pH 7.4. The fractions of the protein peak

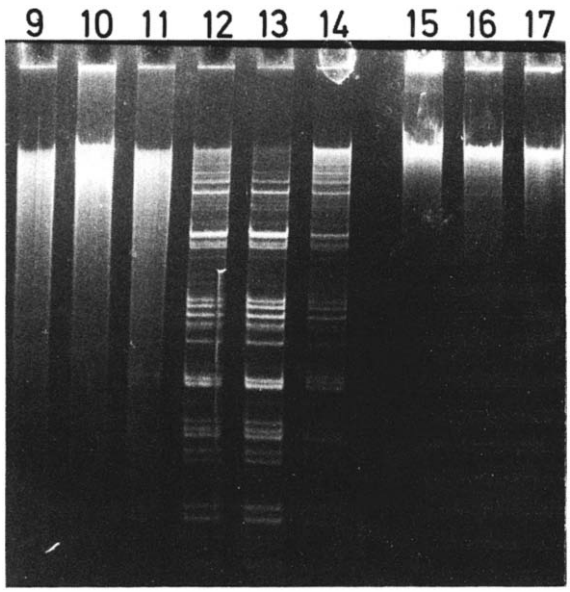

Fig.1. Assay of DEAE-cellulose column. Samples of 10 μl of each fraction (7–8 ml) were assayed for nuclease activity. The reaction mixture (20 μl total vol.) contained 0.7 μg λ-DNA, 10 mM $MgCl_2$ in 25 mM Tris–HCl, pH 7.4. After 30 min reaction was stopped with 10 μl stopper mix (20% sucrose; 2% SDS; 100 mM EDTA, 0.05% bromophenol blue). Samples were electrophorised in a 1% agarose slab gel, 12 h at 40 V. The gel was stained in a 10^{-4}% ethidium bromide solution. The numbers above the slots indicate the fraction number (entire gradient, 65 fractions).

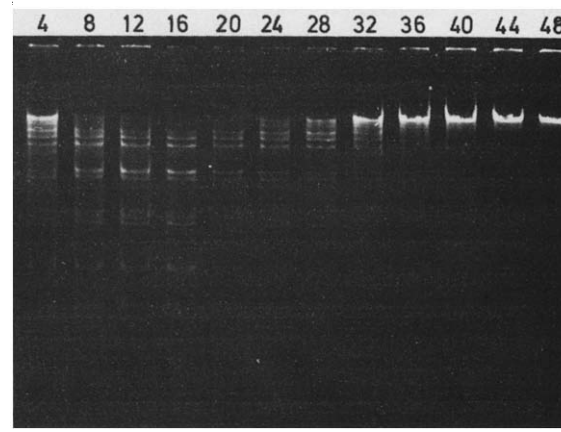

Fig.2. Estimation of optimal $MgCl_2$ concentration. Samples of total vol. 40 μl containing 0.7 μg λ-DNA were incubated at 37°C for 15 min with 4–48 mM $MgCl_2$ in 25 mM Tris–HCl, pH 7.4, stopped with 20 μl stopper mix and electrophorised for 6 h at 70 V as described in fig.1 (numbers indicate mM $MgCl_2$).

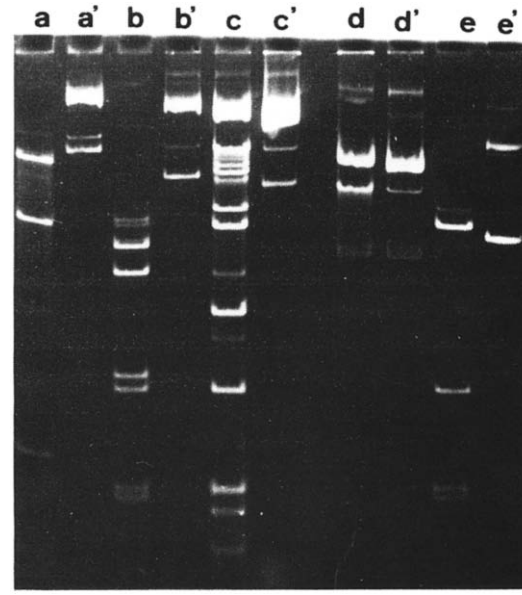

Fig.3. Pattern of digested (a–e) and undigested (a'–e') pSC122 (a), Rsc11 (b), Rsc10 (c), ColE1 (d) and Rsc13 (e). For Rsc-plasmids and cleaved pSF2124 (ColE1-Ap) see ref. [5] and [8], respectively.

were pooled and chromatographed on DEAE-cellulose (Whatman DE52, 2.5 X 15 cm) equilibrated with buffer II (10 mM potassium phosphate, pH 7.4; 10 mM 2-mercaptoethanol). The protein was eluted with a 500 ml linear gradient of 0–0.6 M NaCl in buffer II (fig.1). Fractions were tested for endonuclease activity on λ-DNA by electrophoresis on 1% agarose slab gels, and the gel was stained with ethidium bromide [7]. *Ecl*I eluted between 0.07–0.12 M NaCl.

To determine the pH optimum, portions of 100 μl were dialysed against 50 mM Tris–HCl, pH 6.5 and pH 7.5. The optimal concentrations of $MgCl_2$ and NaCl are 10–20 mM $MgCl_2$ and 50 mM NaCl, respectively (fig.2). Concentrations above 100 mM NaCl strongly inhibit the enzyme activity. KCl hardly influences the reaction up to about 70 mM. The temperature optimum is between 35°C and 40°C. *Ecl*I is stable in the presence of 1 mg/ml BSA at –20°C without appreciable loss of activity for at least six months.

The enzyme does not cleave ColE1, but recognizes three sites in pSF2124 (ColE1-Ap) [8], six in Rsc11 [5] and about 15 in λ-DNA (fig.1).

The digestion pattern obtained after complete cleavage of λ-DNA with *Ecl*I is different from those of *Alu*, *Ava*I, *Bal*, *Bam*, *Bgl*I, *Blu*, *Bum*, *Eco*RI, *Hae*II, *Hae*III, *Hap*II, *Hga*, *Hha*, *Hin*II, *Hin*III, *Hin*f, *Hpa*I, *Kpn*, *Mbo*I, *Mbo*II, *Pst*, *Sal*I, *Sac*I, *Sac*II and *Xma*I [9]. *Ecl*I represents, therefore, most likely a new type II restriction endonuclease.

Acknowledgements

We wish to thank the German collection of Microorganism, Göttingen, for the identification of the *Enterobacter cloacae* strain. We are grateful to Dr R. J. Roberts for providing patterns of λ-DNA digested with the restriction endonucleases mentioned in this paper.

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