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*Bli*I, a restriction endonuclease from *Bacillus licheniformis*

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From *Bacillus licheniformis* a site-specific restriction endonuclease, named *Bli*I, has been purified and characterized. *Bli*I was able to digest λ DNA at pH 9.1 over a wide temperature range (25–65°C). Digestion of λ and φX174 DNAs with *Bli*I produced banding patterns identical to those seen with *Hae*III. Therefore, *Bli*I and *Hae*III endonucleases are isoschizomers.

Restriction enzyme; *Hae*III isoschizomer; *(Bacillus licheniformis)*

1. INTRODUCTION

Site-specific endonucleases have become invaluable tools in research and their use in the analysis and manipulation of nucleic acid molecules has been well documented. Since the discovery of a restriction endonuclease from *Escherichia coli* by Meselson and Yuan [1], more than five hundred restriction endonucleases with a hundred different specificities have been identified [2]. Although a large number of species of microorganisms have been tested for endonucleases, no reports concerning restriction enzyme production by *Bacillus licheniformis* are known. This paper describes the purification and characterization of a type II restriction endonuclease from *B. licheniformis*, named *Bli*I.

2. MATERIALS AND METHODS

The spore-forming strain MP3 used here was originally isolated from soil. The media and procedures used for cultural and physiological characterization were those described by Smith et al. [3]. Cells were grown at 45°C on CESP medium containing 15 g casitone (Difco), 5 g yeast extract (Difco), 3 g soytone (Difco), 2 g peptone technical (Maknur), 0.015 g $MgSO_4 \cdot 7H_2O$, 0.007 g $FeCl_3$ and 0.002 g $MnCl_2 \cdot 4H_2O$ per l, pH 7.2. Cells were harvested by centrifugation at the end of the log phase of growth, then stored frozen at −20°C. 50 g of frozen cells were suspended in 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 0.5 mM DTT (dithiothreitol), 0.1 mM PMSF (phenylmethanesulfonyl fluoride), 10% glycerol, disrupted through a French pressure cell at 12000 lb/inch^2 and centrifuged at 40000 × g for 60 min at 4°C. The removal of nucleic acids and ammonium sulfate fractionation were carried out according to Catterall and Welker [4]. The ammonium sulfate precipitate was dissolved in 20 mM Tris-HCl (pH 7.5), 4 mM 2-mercaptoethanol, 1 mM EDTA, 0.1 mM PMSF, 10% glycerol (buffer A) dialyzed against the same buffer and applied to a DEAE-cellulose column (Whatman DE-52) (2 × 24 cm). The enzyme was eluted with a linear gradient (700 ml) of KCl from 0 to 1 M in buffer A. The active fractions were pooled, concentrated, dialyzed against 20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 7 mM 2-mercaptoethanol, 0.5 mM EDTA (buffer B) and

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applied to a heparin-Sepharose column (Pharmacia) (1.4 × 14 cm) pre-equilibrated with buffer B [5]. The activity was eluted with a linear gradient (300 ml) of NaCl from 0.2 to 1 M in buffer B. Endonuclease active fractions were pooled and concentrated by dialysis for 24 h at 4°C vs buffer B containing 50% glycerol and then stored at −20°C in small portions.

Determination of enzyme activity was routinely performed employing 5–10 μl samples of the enzyme preparation with 1 μg λ DNA in 50 μl of the incubation mixture containing 100 mM Tris-HCl (pH 8.1), 10 mM $MgCl_2$, 7 mM 2-mercaptoethanol. The mixture was incubated at 37°C for 60 min. Reaction was terminated by adding 10 μl of a stop mixture (5% SDS, 25% glycerol, 60 mM EDTA, 0.025% bromophenol blue). Digests were resolved by gel electrophoresis on 1% agarose (BioRad) in 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA. One unit of enzyme activity is defined as the amount of enzyme capable of completely digesting 1 μg λ DNA under specified conditions.

3. RESULTS AND DISCUSSION

In the course of a systematic search for novel restriction enzymes, we tested several strains of the genus *Bacillus*. The crude extract from a strain isolated from soil gave a distinct pattern of bands, on agarose gels after cleavage of λ DNA, characteristic of a restriction enzyme activity. We therefore undertook a purification and characterization of this activity and determined the systematic position of the isolated strain which was found to belong to the species *B. licheniformis* [3,6].

B. licheniformis MP3 was grown, harvested and disrupted as described in section 2. After removal of cell debris by centrifugation, the supernatant was assayed for endonuclease activity on λ DNA. The extract obtained from 1 g frozen cells was found to contain approx. 36000 U. The isolation procedure involves fractionation with ammonium sulfate at 80% saturation and ion-exchange and affinity chromatography. Active fractions eluted from DEAE-cellulose at 0.24–0.31 M KCl and from heparin-Sepharose at 0.72–0.77 M NaCl gave distinct and characteristic gel electrophoretic patterns with λ DNA. The absence of unspecific endonucleases has been verified by incubating 1 μg λ DNA with at least 10 U enzyme for 24 and 48 h. The endonuclease is strictly dependent on Mg^{2+}, and does not require *S*-adenosylmethionine or ATP for activity. The optimal concentration of $MgCl_2$ is 10 mM. The enzyme cleaves DNA efficiently at low salt concentrations (from 10 to 50 mM NaCl). Higher NaCl concentrations decreased the endonuclease activity and no activity was detectable above 200 mM NaCl. Glycerol from 5 to 20% had no apparent effect on the activity. The enzyme was able to digest λ DNA over the pH range 8.1–9.6, with optimal activity at pH 9.1. The endonuclease was found to be active over a wide temperature range, 25–65°C, with a slight decrease in activity at 76°C. The enzyme-catalyzed DNA-cleavage reaction proceeded optimally in 100 mM Tris-HCl (pH 9.1), 50 mM NaCl, 10 mM $MgCl_2$, 7 mM 2-mercaptoethanol at 42°C.

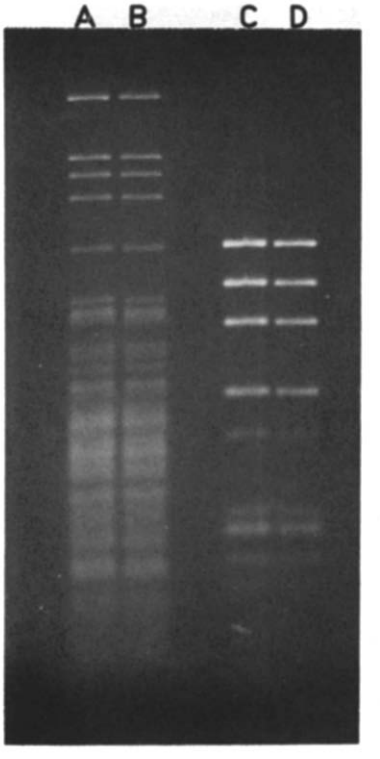

Fig.1. Digestion patterns of λ and φX174 DNAs with *Bli*I and *Hae*III. The DNA fragments were analyzed in a 1.4% agarose gel. (A) *Bli*I on λ DNA; (B) *Hae*III on λ DNA; (C) *Bli*I on φX174 DNA; (D) *Hae*III on φX174 DNA.

The endonuclease activity exhibited by MP3 strain represents the first restriction enzyme found in *B. licheniformis* and should be termed *Bli*I according to the conventional nomenclature [7]. The pattern of restriction fragments produced after digestion of λ and ϕX174 DNAs were indistinguishable from those produced by *Hae*III (fig.1). It appears likely that *Bli*I has the same specificity as *Hae*III recognizing and cleaving the tetranucleotide sequence GGCC. Therefore, *Bli*I and *Hae*III endonucleases are isoschizomers.

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