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THE ISOLATION OF A RESTRICTION ENZYME FROM BORDETELLA PERTUSSIS

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SUMMARY A restriction enzyme was isolated from Bordetella pertussis cells by a single-step purification procedure using chromatography on phosphocellulose. Different DNA molecules were digested with this enzyme; the fragmentation patterns obtained were compared to those obtained after digestion with the Hind III enzyme isolated from Haemophilus influenzae strain Rd. It was concluded that the cleavage site specificities of these enzymes were identical.

INTRODUCTION The restriction and modification of DNA in bacteria is due to the presence of two related enzymes, a restriction endonuclease and a modification methylase (1,2). Restriction endonucleases cleave double-stranded unmodified DNA at specific nucleotide sequences to produce a characteristic set of fragmentation products (3,4). Modification enzymes methylate DNA at or near the sequences recognised by the corresponding restriction endonuclease and thus protect it from degradation. Restriction and modification systems are thought to play a major role in preventing the uptake and expression of foreign DNA by bacteria. Many restriction enzymes, each with a different cleavage site specificity, have now been purified from a wide variety of bacterial strains (4). These enzymes are now widely used in genetic manipulation research (5). One of the possible uses of genetic manipulation is in the construction of "gene banks" of different organisms.

It was found, during the construction of a "gene bank" of Bordetella pertussis, that the DNA of this organism was susceptible to digestion with the Pst I, Eco RI, Bam HI, Hpa I, Hae II, Hae III and Hind II restriction enzymes but was resistant to digestion with the Hind III enzyme. A simple explanation of this result is that Bord. pertussis DNA is methylated at or near the Hind III cleavage sites. It is therefore reasonable to propose that a restriction and

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modification system of similar specificity is present in both Haemophilus influenzae strain Rd and Bord. pertussis. The work reported here supports this hypothesis and describes the isolation of a restriction enzyme from Bord. pertussis.

MATERIALS AND METHODS

Growth of Bord. pertussis. Bordetella pertussis (Wellcome strain 28, serotype 1, 2, 3) was obtained from Dr. P. Novotny (Wellcome Research Laboratories, Beckenham, Kent) and was grown in static culture at 37°C for five days in the growth medium described by Sato et al (6). The cells were recovered by centrifugation at 13,700 x g for 30 min and then stored at -20°C prior to use.

DNA preparations, restriction enzyme digestion and agarose gel electrophoresis

The plasmid pAT153 was obtained from Dr. D. Sherratt (Dept. of Biological Sciences, The University of Sussex) and the plasmid pJDB219 was obtained from Dr. J. Beggs (Plant Breeding Institute, Cambridge). Plasmid DNA was purified from cleared lysates of E. coli HB101 by caesium chloride-ethidium bromide centrifugation (7). Phage strains (λ989, λ641, λ^+) were obtained from Dr. N.E. Murray, (Dept. of Molecular Biology, The University of Edinburgh). Phage λ was grown in E. coli 259, concentrated with polyethylene glycol and purified by caesium chloride equilibrium density gradient centrifugation. Phage λ DNA was isolated from purified phage by phenol extraction. Adenovirus type 2 DNA was purchased from Uniscience Ltd. (Jesus Lane, Cambridge). Restriction enzyme digestions were done in 10mM Tris-HCl pH 7.5, 10mM $MgCl_2$, 10mM 2-mercaptoethanol, 100mM NaCl at 37°C; the reactions were stopped by heating at 70°C for 10 minutes. The digestion products were fractionated by electrophoresis at 20mA for 16 h on 1% (w/v) agarose gels that were prepared and run in 40mM Tris-acetate, pH 8.3, 20mM sodium acetate, 2mM EDTA. The gels were photographed under ultra-violet light after staining with ethidium bromide (1μg/ml).

Restriction enzyme preparation. Bord. pertussis cells (1g wet weight) were thawed and resuspended in 10mM Tris-HCl pH 8.0, 14mM 2-mercaptoethanol (10ml). The cell suspension was sonicated for ten periods of 30sec at 150 W (Ultrasonics Ltd.) at 0°C and then centrifuged at 90,000 x g for 90 minutes. The supernatant was applied directly to a column of phosphocellulose (Whatman P-11, 25cm x 0.7cm diameter) that was pre-equilibrated with 10mM potassium phosphate, pH 7.5, 14mM 2-mercaptoethanol, 0.1mM EDTA, 10% (w/v) glycerol. The column was washed with equilibration buffer (110ml) and the bound proteins then eluted with a linear concentration gradient of KCl to 0.5M (100ml total). The flow rate was 9ml/h and 1.5ml fractions were collected.

RESULTS AND DISCUSSION

The chromatographic profile of the lysed extract of Bord. pertussis cells on phosphocellulose is shown in Fig. 1 and the restriction enzyme assays on selected fractions are shown in Fig. 2. A non-specific nuclease was detected in the unbound protein present in the flow-through from the phosphocellulose column. In addition, an enzyme which cleaved λ^+ DNA into defined fragments

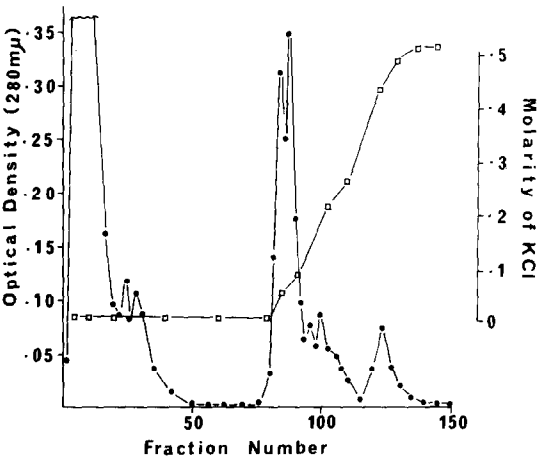

Fig. 1 The chromatographic profile of lysed extracts of Bord. pertussis on phosphocellulose. Bord. pertussis cells were lysed by sonication and the clarified supernatant applied to a column of phosphocellulose as described in Materials and Methods. Bound proteins were eluted from the column with a linear concentration gradient of KCl. ● - ● optical density at 280mμ, □-□ molarity of KCl.

eluted from the phosphocellulose at approx. 0.14M KCl. This enzyme was free of contaminating non-specific nucleases and was therefore not purified further. The enzyme was called Bpe I according to the nomenclature of Smith and Nathans (8).

The fragmentation patterns of different DNA molecules after digestion with Bpe I are shown in Fig. 3 and are compared to the fragmentation patterns obtained after digestion with Hind III. These patterns were identical. In a separate experiment the plasmid pAT153 was digested with Bpe I and then treated with DNA ligase. Religation of the plasmid occurred with high efficiency indicating that digestion with Bpe I produced DNA fragments with cohesive terminii. Bpe I degraded E. coli but not Bord. pertussis chromosomal DNA.

The evidence presented here indicates that Bpe I has a cleavage site specificity identical to Hind III. No evidence was obtained during this work for a Bord. pertussis restriction enzyme similar in specificity to Hind II. It is therefore suggested that Bord. pertussis contains a restriction and modifica-

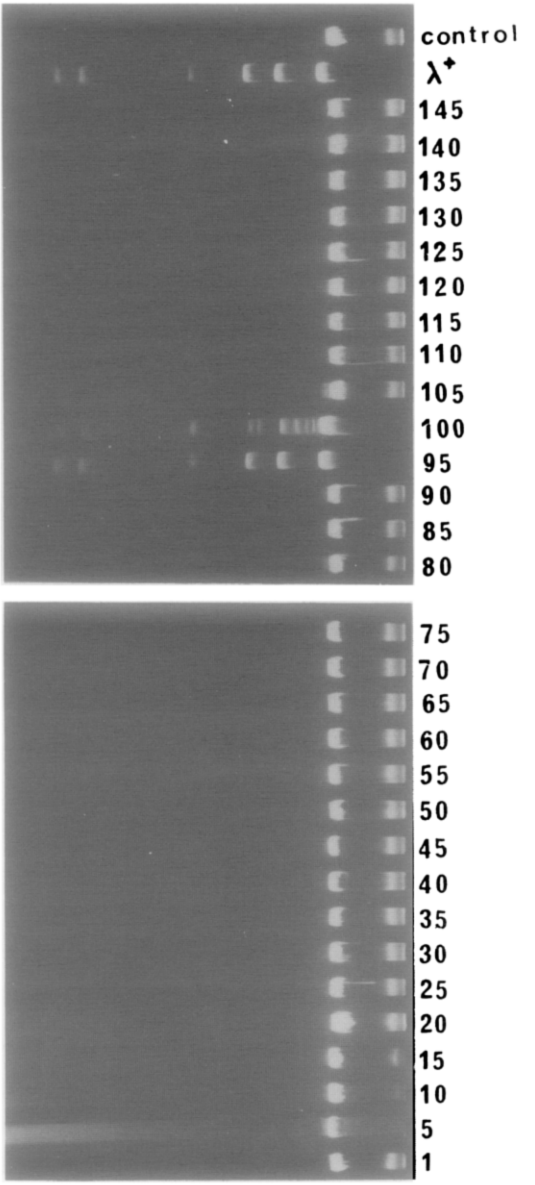

Fig. 2 Assays for enzymic activity in effluent fractions from phosphocellulose. λ^+ DNA (approx. 1µg) was digested with aliquots (10µl) from selected effluent fractions at 37°C for 1 hour and the reaction products analysed by electrophoresis on 1% agarose gels.

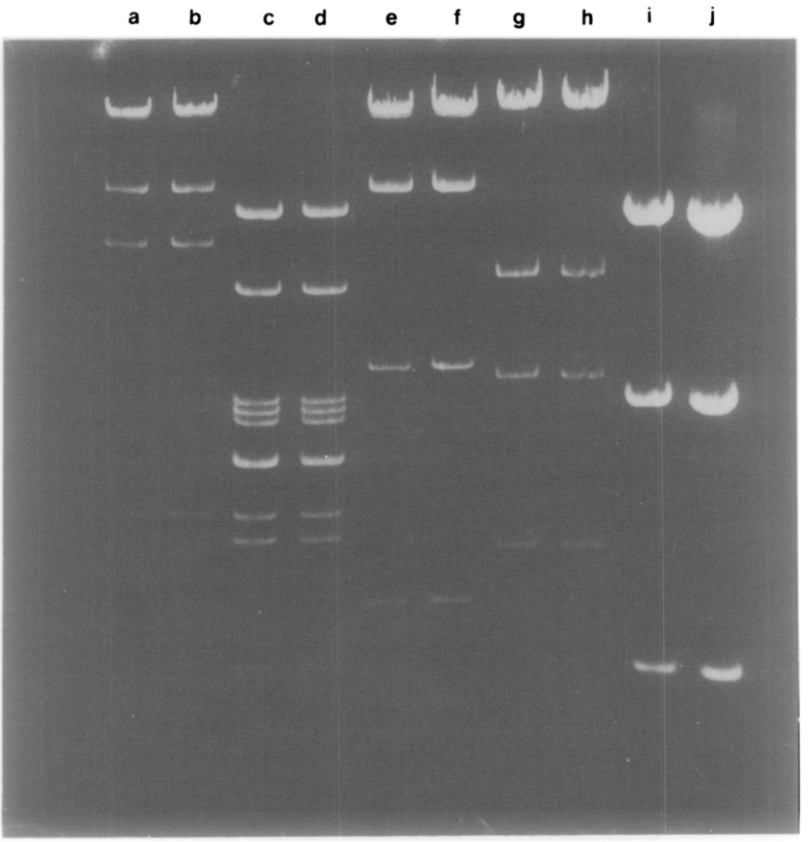

Fig. 3 The digestion of DNA molecules with Bpe I and Hind III. DNA samples were treated with either Hind III (2 units), or Bpe I (20µl, pooled fractions) at 37°C for 2 h and the digestion products analysed by electrophoresis on a 1% agarose gel. Sample order: Lanes (a) λ^+, (c) Ad 2, (e) λ989, (g) λ641 and (i) pJDB219 digested with Hind III; lanes (b) λ^+, (d) Ad 2, (f) λ989, (h) λ641 and (j) pJDB219 digested with Bpe I.

tion system that has a specificity identical to only one of the systems present in Haemophilus influenzae strain Rd. It is of interest to note that the closely related bacterium Bord. bronchiseptica contains a restriction enzyme of similar specificity to Bpe I and Hind III (4).

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