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ISOLATION AND PARTIAL PURIFICATION OF A RESTRICTION ENDONUCLEASE FROM *PSEUDOMONAS OVALIS*

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A restriction endonuclease, PovI, has been isolated and partially purified from *Pseudomonas ovalis* by high speed centrifugation and fractionation on a column of Biogel followed by dialysis and ion exchange chromatography on a phosphocellulose column. The presence of the restriction endonuclease was detected by a simple assay procedure using agarose slahb gel electrophoresis.

Key words: Restriction Endonucleas; Enzymes; *Pseudomonas ovalis.*

INTRODUCTION

The term "restriction endonuclease" was orginally used to designate an endodeoxyribonuclease that was involved in the genetically defined process of host-controlled restriction. The first such enzymes were *Eco B* and *Eco K* isolated from *E.coli* strains Band K [1.2]. Smith and Wilcox in 1970 [3] isolated an enzyme from *Haemophilus influenzae* which differed significantly from *Eco B* and *Eco K,* for while the latter gave a heterogeneous array of products it gave a specific set of discerete DNA fragments. Since the discovery of the first restriction endonuclease, more than 475 restriction ‹endonuclease › have been isolated [4] representing 103 specificities from 356 bacterial strains.

These type II restriction endonucleases recognize and cleave a specific set of base-pairs within a DNA molecule. It is this property of these enzymes that is being widely used in the analysis and restructuring of DNA molecules. Enzymes with new specificities are particularly important in that they increase the range of DNA manipulations that can be performed.

In this study a restriction endonucleases has been isolated and partially purified from a previously unexplored bacterial strain, *Pesudomonas ovalis.*

MATERIALS AND METHODS

Growth and storage of cells. Pseudomonas ovalis, obtained from Dr. M.A. Qadeer, PCSIR Laboratories Lahore was grown in a 12-litre fermenter at 25° in a liquid medium containing 1 % bactotryptone (oxoid), 0.5 % yeast extract (oxoid), and 1 % NaCl. For growth on solid medium nutrient agar (oxoid) was used. Cells were harvested after 10 hr by centrifugation at 5000 rpm for 10 min., and stroed as a frozen cell paste at -20°

Purification of restriction endonuclease. The procedure was carried out while keeping the temperature between O and 4°. The frozen packed cells (10g) were suspended in 20 ml of a buffer containing 0.01M Tris HCl (pH 7.5), 0.01M 2-mercaptoethanol, 20mg lysozyme (sigma), left in stirring position for 1 hr and then disrupted by sonication. The cell lysate was centrifuged at 30,000 rpm for 90 min., in a Beckman ultracentrifuge [5]. The supernatant was made 1.0M NaCl and fractionated on a column (75cmx2.5cm dia.) of Biogel A 0.5m (Biorad) which was eluted with a buffer containing 1.0M NaCl, 0.01M 2-mercaptoethanol, 0.01M Tris HCl (pH 7.5). Fractions containing endouclease activity were combined and dialysed against PC buffer containing 0.01M potassium phosphate (pH 7.4), 0.01M 2-mercaptoethanol, 0.1mM Na_3EDTA, 10 % glycerol, and applied to a phosphocellulose (Whatman p–11) column (17cm x 2.5cm diam.). The column was washed with PC buffer and eluted with a linear gradient of 0-0.1M KCl (200ml) in PC buffer. Fractions with endonuclease activity were combined, concentrated by dialysis against PC buffer and stored at -20°C.

Endonuclease Assays. Column fractions (5ml) were assayed for site specific endonuclease activity by incubating 5μl with 3-5 μg bacteriophage lambda DNA 50 μl 6mM Tris HCl (pH 7.9), 6mM $MgCl_2$, 6mM 2-mercaptoethanol, at 37°Cfor 0.5-1.5 hr. Each reaction was stopped by adding 10μl of a mixture containing 50 % sucrose, 0.2 % bromophenol blue and analysed by electrphoresis on 1.4 % agarose slab gels 20cm x 20cm x 0.3cm [6] at 100 volts for 6hr. The ethidium bromide stained DNA was observed a UV transilluminator and photographed through a filter.

RESULTS AND DISCUSSION

The fractions obtained after high speed centrifugation contained nucleic acids and non-specific nucleases in addition to endonuclease. Biogel chromatography was used to remove these contaminating nucleic acids. Assay of the fractions obtained from the Biogel column revealed the presence of these nucleic acids in fraction nos. 40-48 and that of the restriction endonuclease in fraction nos. 66-80 (Fig. 1).

The fractions with endonuclease activity were combined, dialysed against PC buffer and applied to a phsophocellulose column which has the advantages of selectivity for nucleic acid binding proteins and high capacity. Assay of the fractions obtained from this column clearly indicated the presence of a restriction endonuclease in fractions 66-70 (Fig. 2).

This restriction endonuclease was named Pov 1 according to the nomenclature suggested by Smith and Natjans [7], Fig. 3 shows that the enzyme eluted between 0.4-0.8M KCl.

As seen from Fig. 2 Pov 1 cleaves lambda DNA at least at three places giving four fragments indicating it has at least three cleavage sites on lambda DNA. The fragment pattern produced by Pov 1 appears different from that of the

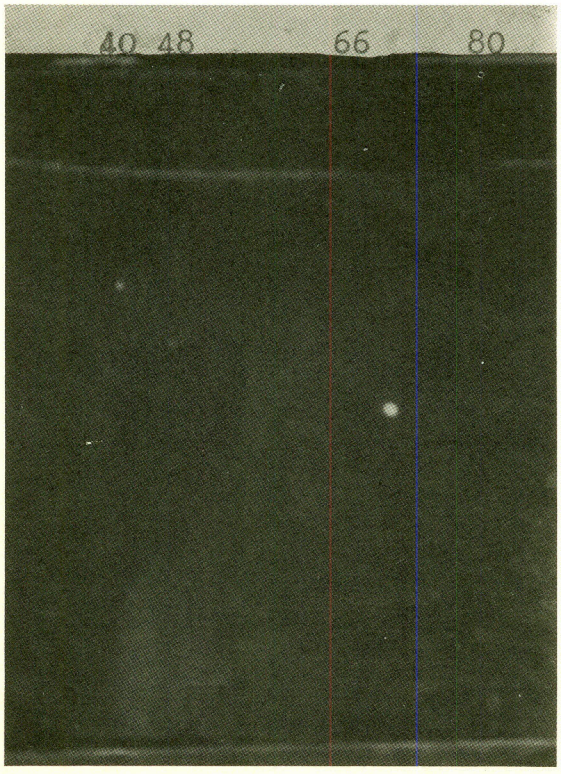

Fig. 1. Assay of fractions from biogel column.

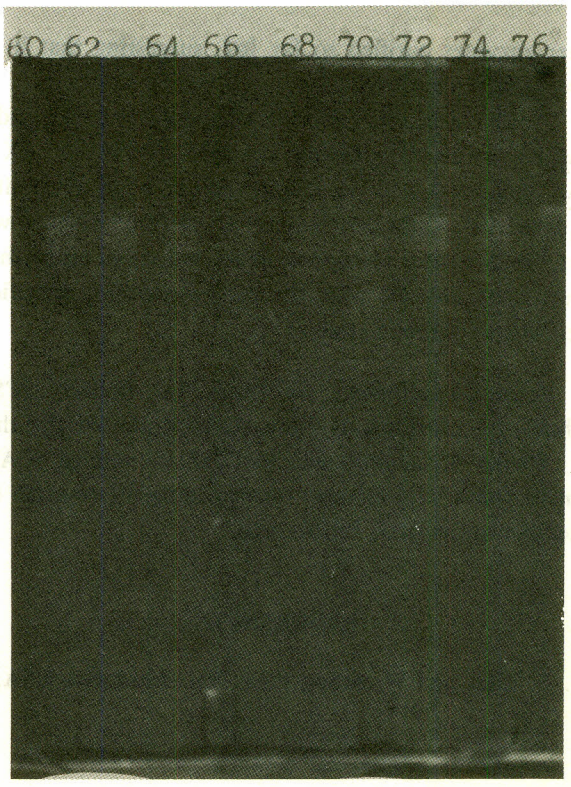

Fig. 2. Assay of fractions from phosphocellulose column.

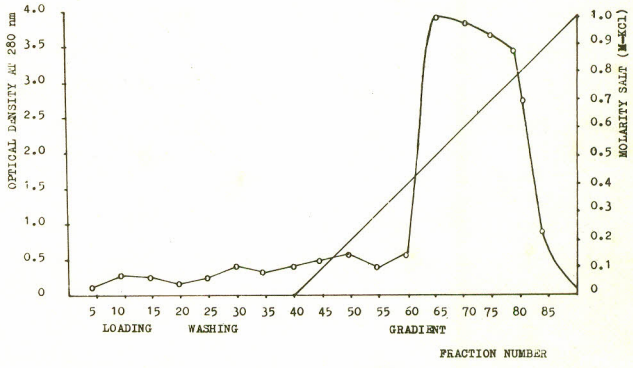

Fig. 3. Elution profile of Pov 1 from phosphocellulose column.

Table 1. Purification of Pov 1. (Protein content was determined according to the method of Bradford [10].

Fraction	Volume (ml)	Protein (mg/ml)	Specific activity (units/mg)	Purification factor
Crude-extract	15	28	$3 \times 10_3$	1.0
Biogel	65	0.8	6×10^4	20
Phospho-cellulose	20	0.24	4.2×10^5	140

other enzymes purified so far [6], suggesting that it is a new restriction endonuclease.

Further characterization Pov 1 should be performed by studying its cleavage pattern on Ad-2, SV-40 and ϕX174 DNA molecules and the determination of its recognition sequence [9]. Only then we can say with confidence whether this is a new restriction endonuclease or an isochizomer (i.e. an enzyme with the same recognition specificity occurring in different strains).

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