Short Communication

A New Restriction Endonuclease from *Bacteroides distasonis* (*BdiI*)[†]Makoto MURAKAMI, Osamu OZAWA,
Tadashi KANEMATSU and Yuzo YAMADA*Research Department, Nissin Sugar Mfg. Co., Ltd.,
4-9-11 Toyosu, Kohto-ku, Tokyo 135, Japan*Laboratory of Applied Microbiology, Department of
Agricultural Chemistry, Shizuoka University,
836 Ohya, Shizuoka 422, Japan

Received October 3, 1989

Type II restriction endonucleases indispensable for gene manipulation and gene analyses have been recovered from procaryotic cells such as those of bacteria and cyanobacteria.¹⁾ We have been studying anaerobic bacteria to find useful restriction endonucleases. During the course of our studies, we have found a new type II restriction endonuclease, designated as *BdiI*, in a strain of *Bacteroides distasonis* (Eggerth and Gagnon 1933) Johnson 1978. This communication describes its purification, properties, and identification of its recognition sequences and cleavage sites on DNA molecules.

Bacteroides distasonis Strain S-7, isolated from human feces, was cultured at 37°C for 48 hr anaerobically in a medium containing 0.4% casamino acids, 0.3% yeast extract, 0.3% Polypepton, 1.0% glucose, 0.15% sodium chloride, 0.1% Tween 80 and 0.04% L-cysteine (pH 7.0).

The restriction endonuclease *BdiI* was purified from cell-free extract of *B. distasonis* Strain S-7 by ammonium sulfate fractionation and combined high performance liquid chromatographies on TSKgel DEAE-5PW Glass, TSKgel HA-1000 Glass and TSKgel G3000SW Glass (Tosoh Corp., Tokyo, Japan). The purified enzyme showed character-

istic digestion patterns of λ DNA (Fig. 1). The enzyme worked best at 45°C in a reaction mixture containing 1.0 μ g λ DNA, 10 mM Tris-HCl (pH 8.2), 7 mM 2-mercaptoethanol, and 7 mM MgCl₂. The enzyme cleaved M13 mp18 RF I, ϕ X174 RF I, SV40 and pBR322 DNAs at 5, ≥ 4 , ≥ 5 and 4 sites, respectively. The enzyme produced four molecules of about 190, 680, 890 and 2600 base pairs, respectively, from pBR322 DNA. These data suggested that the enzyme recognized perfect and imperfect palindromic hexanucleotide sequences 5'-C-T-(A or G)-(T or C)-A-G-3'.²⁾ These four perfect and imperfect palindromic hexanucleotide sequences are found in pBR322 DNA around

Incubation time (hr)
0 0.1 0.25 0.5 1.0 24

Fig. 1. Digestion of λ DNA with the Purified Enzyme (*BdiI*) from *Bacteroides distasonis* Strain S-7.

The purified enzyme (1.0 unit) was incubated for different times from 0 to 24 hr at 37°C in a reaction mixture (50 μ l) containing 1.0 μ g λ DNA, 10 mM Tris-HCl (pH 7.5), 7 mM 2-mercaptoethanol and 7 mM MgCl₂. The reaction was stopped at the time indicated, and the reaction products were analyzed by horizontal agarose gel electrophoresis. The λ DNA was completely digested in 1 hr. In a prolonged incubation for 24 hr, the digestion pattern of λ DNA did not show any remarkable changes. The purified enzyme was considered to be free of other nuclease activities.

[†] Address reprint requests to: Mr. T. Kanematsu, Research Department, Nissin Sugar Mfg. Co., Ltd., 4-9-11 Toyosu, Kohto-ku, Tokyo 135, Japan.

the coordinates 137 (5'-C-T-G-T-A-G-3'), 2738 (5'-C-T-G-T-A-G-3'), 2929 (5'-C-T-A-C-A-G-3') and 3607 (5'-C-T-G-C-A-G-3', recognition sequence of *Pst*I^{*1}). The digestion pattern of pBR322 DNA was not actually disturbed on double digestion with *Pst*I. These results confirmed that the restriction endonuclease from *B. distasonis* Strain S-7 recognizes the perfect and imperfect palindromic hexanucleotide sequences, 5'-C-T-(A or G)-(T or C)-A-G-3'.

Plasmid pBR322 DNA (25 µg, Toyobo) was digested with the purified enzyme and dephosphorylated with bacterial alkaline phosphatase. The dephosphorylated, linear molecules were labeled at the 5'-terminal ends with [γ -³²P]ATP using T4 polynucleotide kinase. The labeled molecules were treated with *Hind*III,^{*2} and the resulting two fragments with 108 and 783 base pairs, isolated by polyacrylamide gel disk electrophoresis, were sequenced by the method of Maxam and Gilbert.³⁾ The smaller fragment revealed gel patterns of 5'-T-A-C-A-G-C-A-T-C-C-A-G-G-G-... from the coordinate 141 in the 3'-direction (coordinate 138 and further) (Fig. 2). The gel patterns of the larger fragment were of 5'-T-G-C-A-G-G-C-A-T-C-G-T-G-G-... from the coordinate 3608 in the 3'-direction (coordinate 3611 and further) (data not shown). The labeled 5'-terminal ends were identified as T for both the fragments.

From the results obtained above, the re-

striction endonuclease from *B. distasonis* Strain S-7 recognizes the perfect and imperfect palindromic hexanucleotide sequences,

Fig. 2. Identification of the Cleavage Ends of the Restriction Endonuclease *Bdi*I.

The two 5'-labeled fragments, cleaved with *Hind*III, from pBR322 DNA were sequenced by the method of Maxam and Gilbert.³⁾ The ladders a, b, c and d represent reaction products of G, A+G, T+C and C, respectively. The smaller fragment revealed gel patterns of 5'-T-A-C-A-G-C-A-T-C-C-A-G-G-G-... from the coordinate 141 in the 3'-direction (coordinate 138 and further). The coordinates between 137 and 142 read 5'-C-T-G-T-A-G-3'.

^{*1} The restriction endonuclease *Pst*I from *Providencia stuartii* 164 recognizes the palindromic hexanucleotide sequence 5'-C-T-G-C-A-G-3' and cleaves pBR322 DNA around the coordinate 3607.

^{*2} The restriction endonuclease *Hind*III from *Haemophilus influenzae* Rd recognizes the palindromic hexanucleotide sequence 5'-A-A-G-C-T-T-3' and cleaves pBR322 DNA around the coordinate 29.

and cuts at the positions indicated by the arrows, producing 5'-tetranucleotide extensions. Since an enzyme which recognizes such perfect and imperfect palindromic hexanucleotide sequences have not been listed hitherto,¹⁾ we here report a new restriction endonuclease, designated as *Bdi*I, from *B. distasonis* Strain S-7. A detailed description of this work will be presented later.

Acknowledgment. We thank Professor Dr. T. Mitsuoka, Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Tokyo, for supplying *Bacteroides distasonis* Strain S-7.

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