Site-specific restriction endonuclease from the filamentous cyanobacterium *Nostoc* sp. MAC PCC 8009

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Received 29 October 1984

We report here the presence of a type-II restriction endonuclease in the filamentous cyanobacterium *Nostoc* sp. MAC PCC 8009. This restriction enzyme, NSp MAC I, is the first reported isoschizomer of *Bgl*II and is very readily purified from non-specific deoxyribonuclease activity in the crude lysate by one round of phosphocellulose column chromatography.

Nsp MAC I Bgl*II isoschizomer* *Cyanobacteria* *Phosphocellulose column chromatography*

1. INTRODUCTION

Nostoc sp. MAC PCC 8009 is a photosynthetic filamentous cyanobacterium isolated from the coralloid roots of a cycad [1]. This strain is capable of regulating synthesis of the two major photosynthetic pigments, phycocyanin and phycoerythrin, in response to changes in light quality, a phenomenon known as chromatic adaptation [2,3]. We are interested in studying the molecular basis of such phenomena in this cyanobacterium and also in the development of a gene cloning system to facilitate transfer of cloned genes for phycobiliproteins from other chromatically non-adapting cyanobacteria. As a preliminary step, we screened this strain for the presence of any site-specific restriction endonuclease which might hinder the development of such a system for gene transfer. Here, we report the presence of an isoschizomer of *Bgl*II enzyme in this cyanobacterium.

2. MATERIALS AND METHODS

Restriction endonucleases *Sau*3A, *Bgl*II, *Bam*HI, *Pvu*I, *Bcl*I and T4 DNA ligase were obtained from Bethesda Research Labs (MD), and digestion of various DNAs with these restriction enzymes were performed according to conditions recommended by the supplier. Lambda DNA and adenovirus type 2 DNA were obtained from Bethesda Research Labs; M13mp10 was purchased from P-L Biochemicals; pUB110 plasmid DNA was prepared as in [4] from *Bacillus subtilis* strain IE6, which was a gift from the Bacillus Genetic Stock Center (Ohio State University).

2.1. *Cell culture and enzyme purification*

Nostoc sp. MAC PCC 8009 was grown in Allen's medium [5] supplemented with 0.1% $NaHCO_3$ in a 7.5 l New Brunswick Scientific Microferm fermentor. The culture was maintained at 37°C under constant illumination from 6 15-W cool white fluorescent lights, aerated with 5% CO_2 and agitated at 200 rpm. Nsp MAC I was purified and assayed essentially as in [6] except the enzyme was concentrated and used after phosphocellulose column chromatography.

2.2. *Ligation and recutting*

Plasmid pUB110 DNA was digested with *Bgl*II

NRC Publication no.23829

Published by Elsevier Science Publishers B.V.
00145793/85/$3.30

and M13mp10 DNA was cleaved with Nsp MAC I. Linearized DNAs were extracted once with phenol before precipitation with ethanol. DNAs were recovered by centrifugation and dissolved in H_2O to a concentration of 200 μg/ml. Equimolar quantities of the two linearized DNAs were mixed and ligated as in [7].

Ligated DNA was recovered after ethanol precipitation by centrifugation and was dissolved in a total of 15 μl H_2O. The sample was divided into 3 5-μl portions: one was subjected to *Bgl*II digestion; the second was subjected to Nsp MAC I digestion, and the third was not further treated.

3. RESULTS

Only one restriction endonuclease activity was detected in this cyanobacterium. Fig.1 shows the phosphocellulose column elution profile of the Nsp MAC I enzyme (assayed against λ DNA). The majority of the enzyme activity is eluted between fractions 41–51, corresponding to an NaCl concentration of 0.3–0.4 M. Judging by the sharpness of the DNA bands in the lanes of the agarose gel corresponding to these fractions, the eluted enzyme appears to be free of contaminating nonspecific nucleases which are eluted in the flow-through fraction. Prolonged (up to 24 h) digestion of λ DNA with enzyme from these fractions produced DNA bands in the agarose gel with the same sharpness as those obtained after 1 h of digestion (not shown). The multiple bands in the high-M_r areas in lanes corresponding to fractions 29–39 and 53–67 are readily interpretable as incomplete digestion products. This enzyme cleaves λ DNA into 6 fragments with lengths of approx. 22.0, 13.29, 9.69, 0.65, 0.4 and 0.01 kbp (the two smallest fragments are detectable in polyacrylamide gels). These fragment sizes are in good agreement with the published values of *Bgl*II-digested λ DNA [8].

Fig.2 depicts digestion of λ DNA with *Sau*3A (lane 2), *Bgl*II (lane 3), *Bgl*II and Nsp MAC I (lane 4) and Nsp MAC I (lane 5). It is evident that *Sau*3A (with recognition sequence of GATC) displays a different digestion profile to that of Nsp MAC I. Not only does *Bgl*II-digested λ DNA show the same fragment profile as Nsp MAC I, but the same profile is also obtained with λ DNA subjected to double digestion with *Bgl*II and Nsp MAC I. A similar result was obtained by using adenovirus type 2 DNA as a substrate (fig.3). Other enzymes with the same internal tetranucleotide (GATC) in their hexameric palindrome recognition sequences, such as T↓GAT$^{m}_{C}$CA (*Bcl*I, lane 2), C↓G$^{m}_{A}$TCG (*Pvu*I, lane 3) and G↓GATC$^{m}_{C}$ (*Bam*HI, lane 4), produce different fragment profiles from those of *Bgl*II and Nsp MAC I.

Further demonstration of the identity of the recognition sequence of Nsp MAC I and *Bgl*II is illustrated in fig.4. Lanes 2 and 6 depict *Bgl*II linearized plasmid pUB110 DNA and Nsp MAC I-linearized M13mp10 DNA, respectively. Successful ligation is evidenced by the disappearance

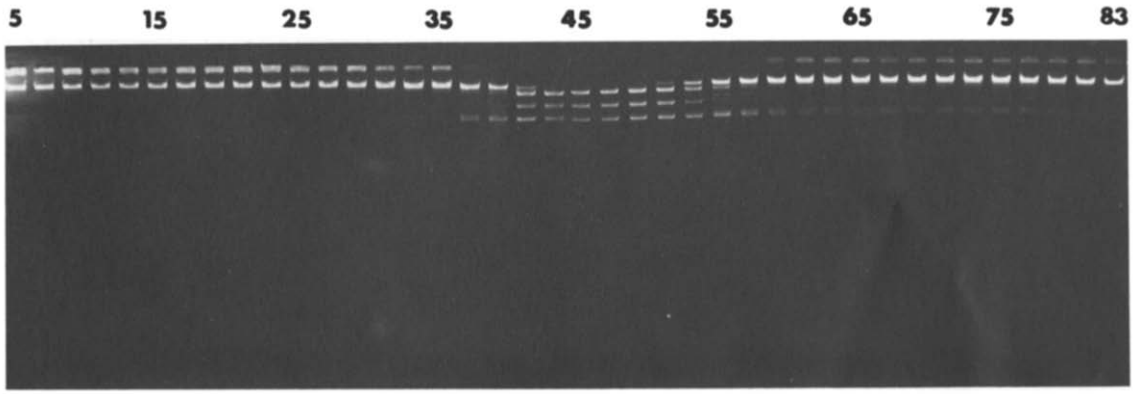

Fig.1. Phosphocellulose column elution profile of Nsp MAC I assayed against λ DNA. Numbers on top of the agarose gel photograph denote fraction numbers. Fractions preceding fraction 5 and subsequent to fraction 83 are not included.

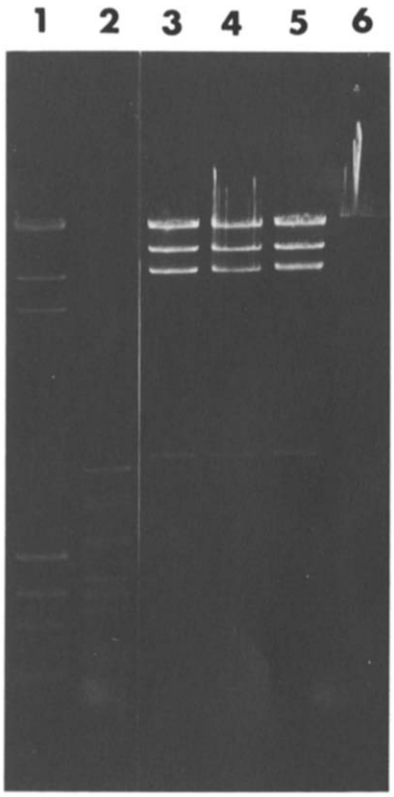

Fig.2. Digestion pattern obtained with λ DNA. Lane 1, size markers (λ *Hin*dIII and φX174 *Hae*III digests); lane 2, *Sau*3A digest; lane 3, *Bgl*II digest; lane 4, *Bgl*II-Nsp MAC I double digest; lane 5, Nsp MAC I digest; lane 6, undigested λ DNA.

of the component DNAs and the appearance of higher-M_r fragments (lane 4). Upon redigestion of the ligated DNA with *Bgl*II (lane 3) and Nsp MAC I (lane 5), the two component fragments are recovered. The fragments regenerated by *Bgl*II and Nsp MAC I digestion are identical in size to the two original input fragments before ligation. Such results can be expected if Nsp MAC I not only recognizes the same sequences but also cleaves at the same sites. We conclude that the recognition sequence of Nsp MAC I is A↓GATCT and that this enzyme is an isoschizomer of *Bgl*II.

4. DISCUSSION

*Bgl*II is a widely used restriction endonuclease in sequence studies and in in vitro manipulation of

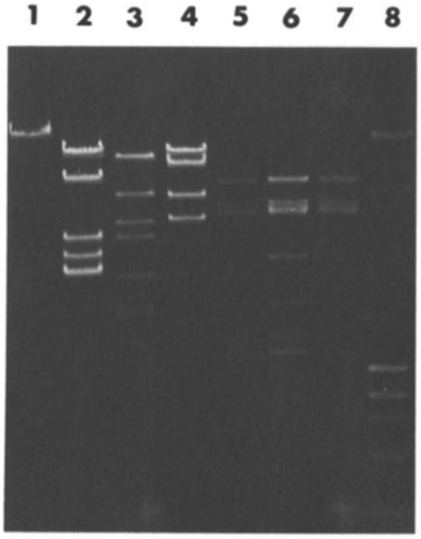

Fig.3. Digestion pattern obtained with Ad-2 DNA. Lane 1, undigested Ad-2 DNA; lane 2, *Bcl*I digest; lane 3, *Pvu*I digest; lane 4, *Bam*HI digest; lane 5, *Bgl*II digest; lane 6, *Bgl*II-Nsp MAC I double digest; lane 7, Nsp MAC I digest and lane 8, size markers (λ *Hin*dIII and φX174 *Hae*III digests).

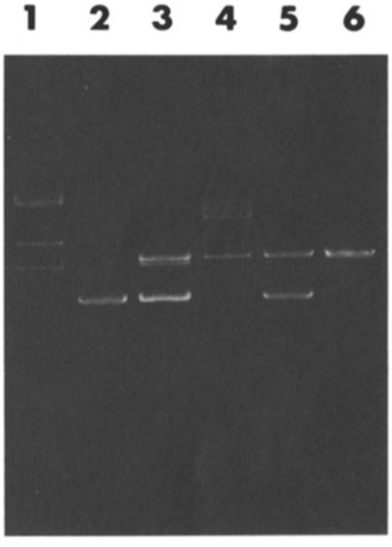

Fig.4. Ligation and recut of *Bgl*II-linearized pUB110 DNA and Nsp MAC I-linearized M13mp10 DNA. Lane 1, size markers (λ *Hin*dIII digest); lane 2, *Bgl*II-digested pUB110 DNA; lane 3, *Bgl*II digest of ligated DNA; lane 4, ligation products of pUB110-*Bgl*II and M13mp10-Nsp MAC I fragments; lane 5, Nsp MAC I digest of M13mp10 DNA.

DNA. As far as we are aware, Nsp MAC I is the first isoschizomer of *Bgl*II to be described. Many isoschizomers have been found for other restriction enzymes (e.g., *Hin*dIII, *Xho*I, *Sal*I and *Ava*I and II [9]). The reason for the paucity of *Bgl*II isoschizomers is unclear.

Nsp MAC I offers two advantages over *Bgl*II. Firstly, it is the only restriction endonuclease present in this cyanobacterial strain, thus eliminating the possibility of cross contamination by a second restriction enzyme present within the same cell. (*Bacillus globigii*, from which *Bgl*II is derived, contains a second enzyme, *Bgl*I [10,11].) Secondly, Nsp MAC I is very readily purified from contaminating non-specific nucleases which will not bind to phosphocellulose under the conditions described. The resulting enzyme preparation is suitable for most purposes after only one round of column chromatography. The yield of Nsp MAC I is approx. 1000 units/g cells (fresh weight).

The presence of Nsp MAC I in this strain could seriously lower the efficiency of introduction of foreign DNA that carries nucleotide sequence susceptible to cleavage by Nsp MAC I. One of us (R.H.L.) is currently attempting to generate mutants of this strain, defective in the production of this enzyme through mutagenesis, to afford protection of transforming DNAs against possible degradation within the cell.

ACKNOWLEDGEMENTS

This work was supported in part by a research grant to R.H.L. from the Advisory Research Committee of Queen's University.

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