The size of the chromosome of *Azotobacter chroococcum*

Adhar C. Manna and H. K. Das

Author for correspondence: H. K. Das. Tel: +91 11 661044. Fax: +91 11 6865886.
e-mail: uunet!shakti!vikram!jnuniv!hiren

Genetic Engineering Unit,
Centre for Biotechnology,
Jawaharlal Nehru University,
New Delhi – 110067, India

The DNA of *Azotobacter chroococcum* M4 was digested with rare-cutter restriction endonucleases *AseI* and *DraI* and restriction fragments were separated by pulsed-field gel electrophoresis. The chromosome size was estimated to be around 5300 kb by summing the sizes of the resolved restriction fragments.

Keywords: *Azotobacter chroococcum*, chromosome size, pulsed-field gel electrophoresis

Azotobacter vinelandii and *Azotobacter chroococcum* are aerobic, nitrogen-fixing bacteria with multiple chromosomes: *A. vinelandii* UW can contain 40–80 chromosome copies (Sadoff *et al.*, 1979; Nagpal *et al.*, 1989). The chromosome size of *A. vinelandii* has been determined to be around 4500 kb by pulsed-field gel electrophoresis (PFGE) (Manna & Das, 1993). The *E. coli* chromosome is about 4700 kb in size (Smith *et al.*, 1987).

PFGE allows the resolution of large DNA molecules and permits the accurate determination of chromosome size. We report here the use of this technique to estimate the size of the chromosome of *A. chroococcum* M4, a wild-type strain isolated from the rhizosphere of maize plants at the Indian Agricultural Research Institute, New Delhi (Apte & Shende, 1981).

Growth of cells, embedding, lysis and digestion with restriction endonucleases for PFGE were done as described by Manna & Das (1993). The DNA bands from agarose gels were transferred to nitrocellulose membranes according to Sambrook *et al.* (1989). The DNA fragment which was to be used as probe for hybridization was eluted from a low-melting-point agarose pulsed-field gel (1.0%, w/v). The fragment was radiolabelled by using random primers (Feinberg & Vogelstein, 1983) and Southern hybridization was performed as described in Sambrook *et al.* (1989).

Accurate measurement of genome size by PFGE relies on the ability to resolve the fragments upon electrophoresis; this requires restriction endonucleases that cleave infrequently. The genomes of *Azotobacter* species have G + C contents in the range 64.8 to 66.7 mol% (Robson *et al.*, 1984); thus enzymes that recognize only A–T sequences may cleave infrequently. *A. chroococcum* DNA was digested with *AseI*, *DraI*, *SspI* and *SpeI* (New

England Biolabs) and the fragments were separated by PFGE. *SspI* and *SpeI* yielded too many fragments of less than 150 kb (data not shown). *AseI* and *DraI* generated 7 and 20 bands, respectively (Fig. 1a). When undigested *A. chroococcum* DNA was subjected to PFGE, all the DNA remained in the well during electrophoresis (Fig. 1a, lane 1), indicating that the *A. chroococcum* genome was intact, and no shearing or degradation of DNA occurred during the preparation, storage or electrophoresis. When *A. vinelandii* chromosomal DNA was digested with *AseI* or *DraI* and separated by PFGE, more than 30 fragments were visible (unpublished results), which had very little similarity with those from *A. chroococcum*.

The sizes of the bands of *AseI* and *DraI* digest of *A. chroococcum* DNA, estimated using yeast chromosomes and λ DNA concatemer markers (New England Biolabs), are given in Table 1. Sizes of large fragments were determined by graphical analysis of two-point linear interpolation (Heath *et al.*, 1992). Logarithmic interpolation was used for smaller fragments.

The intensity of UV fluorescence of ethidium-bromide-stained bands was determined by laser scanning (Pharmacia-LKB, Ultra-Scan XL) of polaroid photograph negatives. The fluorescence intensity of bands composed of linear DNA fragments in equimolar concentrations should increase with their size. Based on this principle, we plotted intensity (area under the peaks in the tracing) versus size (kb) of the fragments generated by *AseI* and *DraI* (data not shown). We inferred that band 9 of *DraI* digests comprised two DNA fragments of about 125 kb, whereas all the other *DraI* bands comprised a single fragment. For the *AseI* digest, we concluded that bands 2, 4 and 6 comprised two DNA fragments each (780, 445 and 140 kb, respectively) and that band 5 consisted of three DNA fragments of about 350 kb.

Complete digestion of *A. chroococcum* DNA with *AseI* and *DraI* was confirmed by Southern hybridization using the

Abbreviation: PFGE, pulsed-field gel electrophoresis.

Fig. 1. PFGE of *A. chroococcum* M4 DNA. Electrophoresis was in 1% agarose (PFGE grade, Sigma) in 50 mM Tris/borate, 0.1 mM EDTA, pH 8.0, at 200 V and 12–14 °C. Pulse times were 5 s to 120 s for 20 h for the experiment shown in lane 1, and 5 s to 100 s for 18 h followed by 120 s for 4 h for all others. (a) Lane 1, undigested *A. chroococcum* DNA; lane 2, *AseI* digest; lane 3, *DraI* digest; lane 4, low-range PFG marker DNA; lane 5, *Saccharomyces cerevisiae* YPH80 chromosomes. (b) Autoradiogram of Southern hybridization blot of lanes 2 and 3 of (a) using the 1690 kb *DraI* DNA fragment as probe. (c) Schematic interpretation of bands in lanes 2–5 of (a). Doublets or triplets are indicated with thicker bands. Sizes are given in kb.

1690 kb *DraI* DNA fragment as α - 32 P-labelled radioactive probe. Among the fragments generated by *DraI* digestion, only the 1690 kb fragment hybridized (Fig. 1b, lane 3). The 930 kb and 780 kb bands of *AseI* digests also hybridized, suggesting that these had originated from the 1690 kb *DraI* fragment. The intensity of the signals in the autoradiogram of *AseI* lane, as determined by laser scanning, confirmed the presence of two DNA fragments in band 2 of *AseI* digests. Thus we estimate the size of the *A. chroococcum* chromosome to be 5296 kb based on the fragments generated by *DraI* digestion, and 5305 kb based on *AseI* digestion.

Our estimate of the size of the *A. chroococcum* chromosome is larger than that of Robson *et al.* (1984), who used two-dimensional agarose gel electrophoresis to obtain size estimates of 1780 kb for *A. chroococcum* CW8 and 1940 kb for *A. chroococcum* MCD-1. In their experiments the DNA was digested with the first restriction enzyme (*EcoRI* for CW8 and *BstEII* for MCD-1), electrophoresed in one direction and then digested *in situ* with the second enzyme (*BstEII* for CW8 and *XbaI* for MCD-1), and the fragments electrophoresed in the second direction. The enzymes

Table 1. Sizes of fragments of the *A. chroococcum* chromosome produced by *DraI* and *AseI*

Fragment no.	Fragment size (kb)*	
	<i>DraI</i> digestion	<i>AseI</i> digestion
1	1690 $\pm$ 10	930 $\pm$ 10
2	880 $\pm$ 10	780 $\pm$ 15
3	700 $\pm$ 10	780 $\pm$ 15
4	485 $\pm$ 5	510 $\pm$ 10
5	280 $\pm$ 5	445 $\pm$ 5
6	190 $\pm$ 5	445 $\pm$ 5
7	175 $\pm$ 5	350 $\pm$ 5
8	145 $\pm$ 5	350 $\pm$ 5
9	125 $\pm$ 4	350 $\pm$ 5
10	125 $\pm$ 4	140 $\pm$ 4
11	85 $\pm$ 3	140 $\pm$ 4
12	83 $\pm$ 3	85 $\pm$ 3
13	63 $\pm$ 2	
14	60 $\pm$ 2	
15	50 $\pm$ 2	
16	48 $\pm$ 2	
17	35 $\pm$ 1	
18	25 $\pm$ 1	
19	18 $\pm$ 1	
20	13 $\pm$ 1	
21	11 $\pm$ 1	
Total size of chromosome (kb)	5296	5305

* Means of five different experiments, $\pm$ SE.

used were not infrequent cutters. Many of the fragments were probably not resolved and hence the estimate of the total size was low.

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