J. Genet., Vol. 76, Number 1, April 1997, pp. 55–60. © Indian Academy of Sciences

The *Azotobacter vinelandii* chromosome

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MS received 25 July 1996; revised received 1 October 1996

Abstract. The chromosome of *Azotobacter vinelandii* was digested with the restriction endonucleases *Spe*I (5′-ACTAGT), *Dra*I (5′-TTTAAA) and *Ase*I (5′-ATTAAT) and the products were separated by pulsed-field gel electrophoresis. The sum of the sizes of the restriction fragments comes to around 4·5 megabasepairs. Our earlier studies had revealed the presence of about 80 copies of *nifH*, *nifD*, *nifK* and *leuB* genes in a log-phase cell of *A. vinelandii*. To determine whether there are multiple identical chromosomes in *A. vinelandii* or one large chromosome with identical segments joined in tandem, we have subjected gamma-irradiated DNA of *A. vinelandii* and *Escherichia coli* to pulsed-field gel electrophoresis. The results suggest that *A. vinelandii* chromosomes contain multiple identical chromosomes of about the same size as that of *E. coli*.

Keywords. Gamma irradiation; pulsed-field gel electrophoresis.

1. Introduction

Azotobacter vinelandii, the aerobic diazotroph, is a novel prokaryote. A log-phase cell of *A. vinelandii* UW contains as much as $1{\cdot}35 \times 10^{-13}$ g of DNA, while a log-phase cell of *Escherichia coli* K12 W3110 contains only $3{\cdot}4 \times 10^{-15}$ g of DNA (Nagpal *et al.* 1989). How is this huge amount of DNA organized in *A. vinelandii*?

Complexity of DNA from *A. vinelandii*, as determined by renaturation kinetics, has been found to be similar to that from *E. coli* (Sadoff *et al.* 1979; Nagpal *et al.* 1989). In addition, each of the genes *leuB*, *nifH*, *nifD* and *nifK* has been demonstrated to be present in about 80 copies in a cell of *A. vinelandii* (Nagpal *et al.* 1989).

The *A. vinelandii* chromosome had been digested with rare-cutting restriction endonucleases *Swa*I (5′-ATTTAAAT), *Pme*I (5′-GTTTAAAC) and *Pac*I (5′-TTAATTAA) and the resulting DNA fragments were separated by pulsed-field gel electrophoresis. The sizes of these DNA fragments, when added up, yielded the notional size of the *A. vinelandii* chromosome to be 4550, 4490 and 4440 kbp for digests with *Swa*I, *Pac*I and *Pme*I respectively (Manna and Das 1993). The size of the *E. coli* chromosome has been found to be 4·7 megabasepairs (Smith *et al.* 1987; Kohara *et al.* 1987).

Subsequently, Maldonado *et al.* (1994) reported the size of the *A. vinelandii* chromosome to be 4580 kbp based on the size of fragments obtained by digestion with *Swa*I (5′-ATTTAAAT). They had also digested the chromosome with *Spe*I (5′-ACTAGT), but the size of the chromosome deduced on the basis of this digestion came to 4795 kbp (Maldonado *et al.* 1994). They have attributed this anomaly to variation intrinsic to the technique of pulsed-field gel electrophoresis.

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In our hands, however, the sizes of the fragments obtained from the *A. vinelandii* chromosome by digestion with *Spe*I (5′-ACTAGT) when added up yielded a value of 4558 kbp. We have also verified the size by digestion with two other six-cutter enzymes, *Dra*I (5′-TTTAAA) and *Ase*I (5′-ATTAAT). These results are reported here.

The important point, nevertheless, is that none of the above information distinguishes between the two possibilities, (i) an *A. vinelandii* cell contains either a single huge chromosome of a size about 80 times that of *E. coli* composed of 80 identical genomes joined in tandem, or (ii) it contains 80 identical separate chromosomes of about the size of the *E. coli* chromosome.

Gamma irradiation is known to produce double-strand breaks (DSB) in DNA. The dose of irradiation can be so adjusted to yield a significant number of molecules with only one break, converting circular molecules into full-length linear ones, which would form a band of monodisperse-size molecules on pulsed-field gel electrophoresis. Under this condition some circular molecules would remain intact and would be left over in the sample block, while some other molecules would suffer multiple DSBs generating random-sized DNA fragments, which would appear as a smear in the gel. A method was described based on this principle to determine the length of chromosome of different species of *Mycoplasma* by pulsed-field gel electrophoresis (Neimark and Lange 1990). The size range found was between 684 and 1315 kbp.

We have adapted this method to look at the chromosomes of *A. vinelandii*. The *E. coli* chromosome which has been found to be around 4·7 megabp (Kohara *et al.* 1987; Smith *et al.* 1987) has been used as a standard.

2. Materials and methods

2.1 *Bacterial strains*

Azotobacter vinelandii UW (non-gummy derivative of the wild-type strain) and *Escherichia coli* HB101 (an *E. coli* K12 × *E. coli* B hybrid) were obtained from W. J. Brill, University of Wisconsin, Madison, USA, and H. B. Boyer, University of California, School of Medicine, San Francisco, USA, respectively.

2.2 *Preparation of DNA for pulsed-field gel electrophoresis*

Bacteria were grown, plugs were prepared, and cells in the plugs were lysed as described earlier (Manna and Das 1993). The plugs were washed three times in $T_{10}E_{0.1}$ (10 mM Tris-Cl, pH 7·6, and 0·1 mM EDTA, pH 8·0) and stored in $T_{10}E_{0.1}$ in ice. BioRad CHEF-DR II electrophoresis equipment was used at 14° C.

2.3 *Digestion with restriction endonucleases*

The endonucleases *Spe*I, *Dra*I and *Ase*I were obtained from New England Biolabs. The agarose plugs were equilibrated with the digestion buffer specified by New England Biolabs for each endonuclease at 4° C for 20 min. Digestions were then carried out for 8–12 h. Termination was achieved with 100 mM EDTA, *p*H 8·0.

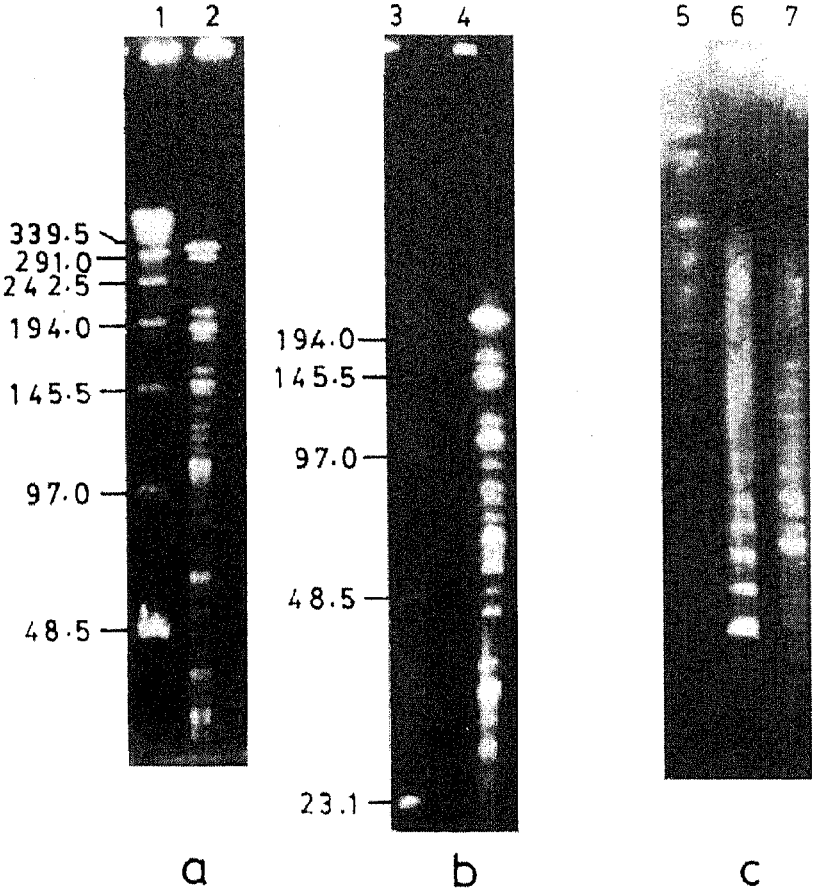

Figure 1. Pulsed-field gel electrophoresis of *A. vinelandii* UW DNA digested with three six-base-cutter endonucleases, *Ase*I, *Dra*I and *Spe*I. The electrophoresis conditions were as follows: elecrophoresis was in 1% agarose at 200 V and 14°C; pulse times were 20 sec to 100 sec for 25 h for panels a and b, and 50 sec for 15 h and 120 sec for 10 h for panel c. Lane 1, lambda ladder; lane 2, genomic DNA digested with *Ase*I; lanes 3 and 6, low-range PFG markers; lane 4, genomic DNA digested with *Dra*I; lane 5, yeast YPH 80 chromosomes; lane 7, genomic DNA digested with *Spe*I.

3. Results and discussion

3.1 *Analysis of the DNA fragments obtained by digestion of the A. vinelandii chromosome with restriction endonucleases*

Results of pulsed-field gel electrophoresis of the fragments obtained by digestion with *Ase*I, *Dra*I and *Spe*I are presented in figure 1. The large DNA fragments were optimally separated using long-run pulse times, whereas the smaller DNA fragments were separated using short pulse times.

The mobility of the DNA fragments was compared with those of yeast chromosomes, lambda DNA concatamers and low-range PFG markers (a mixture of lambda concatamers and *Hin*dIII digest of lambda DNA). Sizes of the fragments were determined by two-point logarithmic interpolation.

The intensity of the ultaviolet fluorescence of the ethidium-bromide-stained bands was determined by laser scanning (Pharmacia–LKB Ultrascan XL) of the Polaroid

Table 1. Sizes of fragments of the *A. vinelandii* chromosome produced by the enzymes *Spe*I, *Ase*I and *Dra*I.

Band no.	Fragment size (kbp)					
	SpeI[a]	Error	*AseI*[a]	Error	*DraI*[a]	Error
1	570	±20[c]	316(2)[b]	±5	324	±6
2	520	±10	288	±5	302(2)	±6
3	485	±10	209	±5	245·5(2)	±6
4	425	±5	190·5(2)	±4	174(3)	±5
5	335	±5	182	±4	162(3)	±5
6	275(2)	±5	158·5	±4	138(4)	±5
7	250	±4	151(2)	±4	123	±4
8	220	±4	144·5	±4	114·5	±4
9	200(4)	±3	132	±4	109·5	±4
10	82	±2	126	±3	93	±4
11	60	±2	120	±3	85	±4
12	55	±2	115	±3	83	±4
13	38	±2	112(2)	±3	79	±4
14	35	±1	110(2)	±3	71(3)	±4
15	29	±1	107(2)	±3	66	±2
16	25	±1	100(2)	±3	63	±2
17	24	±1	91	±3	58	±2
18	22	±1	83	±2	55(2)	±2
19	18	±1	72	±2	52	±2
20	15	±1	63(3)	±2	50	±2
21			60	±2	45	±2
22			50(2)	±2	40(2)	±2
23			44	±2	30	±1
24			33(2)	±2	28	±1
25			30	±2	25(2)	±1
26			25(2)	±2	22	±1
27			23(2)	±2	16	±1
28			20	±1	15	±1
29			17(2)	±1	12	±1
30			11	±1	10	±1
31			9·5	±1	9	±1
32			6·5	±1	7·5[d]	
33			3·0	±1	6·5[d]	
34			2·0(2)	±1	3·0[d]	
35			1·0	±1	2·5[d]	
36			0·5	±1	0·8[d]	
37					0·2[d]	
Total:	4558·0		4566·0		4585·0	

[a]Sizes were the average obtained from five different experiments.
[b]Number of fragments per band as determined by densitometry scanning.
[c]Error, largest deviation from the average among the individual values.
[d]Sizes of fragments were determined by comparison with DNA fragments of the *nif* cluster (Jacobson *et al.* 1989).

negatives. The intensity values (area under the peak in the tracing) when plotted against the size of the DNA fragments allowed us to pick up the bands that had multiple DNA fragments and also estimate the number of fragments (*cf.* Manna and Das 1993). The estimated sizes of the fragments are presented in table 1. The results unambiguously

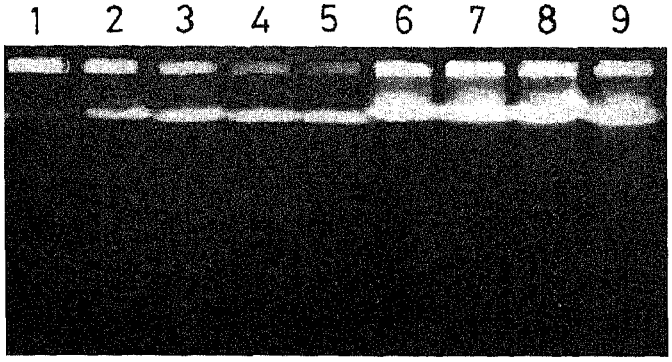

Figure 2. Pulsed-field gel electrophoresis of gamma-irradiated DNA. Plugs containing *A. vinelandii* UW DNA were gamma irradiated with 20 gray (lane 1), 30 gray (lane 2), 60 gray (lane 3), 90 gray (lane 4), and 120 gray (lane 5), and plugs containing *E. coli* HB101 DNA with 30 gray (lane 6), 60 gray (lane 7), 90 gray (lane 8), and 120 gray (lane 9). Electrophoresis was in 0·8% agarose in 0·5 × TBE (44 mM Tris, 44 mM boric acid, 1·2 mM EDTA, pH 8·0) at 14°C at 80 V with pulse times 100 sec for 15 h, 120 sec for 10 h and 150 sec for 10 h.

confirm our earlier inference that the sum of the sizes of the fragments of the chromosome of *A. vinelandii* is around 4·5 megabasepairs.

3.2 *Pulsed-field gel electrophoresis of A. vinelandii chromosome after gamma irradiation*

The inference drawn in the preceding section is based on the assumption that the *A. vinelandii* genome comprises multiple identical chromosomes and not a single chromosome with multiple identical segments joined in tandem. These alternatives were examined next.

Plugs containing lysed *A. vinelandii* UW and *E. coli* HB101 were gamma-irradiated for different lengths of time from a ^{60}Co source and subjected to pulsed-field gel electrophoresis (figure 2). The result offers unequivocal demonstration that the size of the *A. vinelandii* chromosome is almost the same (slightly smaller) as that of *E. coli*.

We can therefore conclude that the size of 4·5 megabase arrived at by the addition of the sizes of restriction fragments (Manna and Das 1993; Maldonado *et al.* 1994) produced by the eight-cutter restriction endonucleases *Swa*I, *Pme*I and *Pac*I indeed represents the size of the *A. vinelandii* chromosome. The results thus indicate that log-phase *A. vinelandii* cells contain about 80 copies of chromosome slightly smaller in size than the *E. coli* chromosome.

Acknowledgements

This work was supported by the Department of Biotechnology, Government of India. A.C.M. is obliged to the University Grants Commission for a fellowship.

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