The Genomes of *Desulfovibrio gigas* and *D. vulgaris*

By JOHN R. POSTGATE,* HELEN M. KENT, ROBERT L. ROBSON AND
JULIAN A. CHESSHIRE

ARC Unit of Nitrogen Fixation, University of Sussex, Brighton BN1 9RQ, UK

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Two-dimensional electrophoresis of sequential double-restriction digests showed that the genome of *Desulfovibrio gigas* comprised 1.63×10^6 bp (1.09×10^9 Dal) of DNA; an ammonia-limited chemostat population possessed an average of nine genomes per cell and a multiplying batch culture possessed ~ 17 genomes per cell. The genome size of *D. vulgaris* (Hildenborough) was 1.72×10^6 bp (1.14×10^9 Dal); a population from an ammonia-limited batch culture contained four genomes per cell. Control digestions and analyses with *Escherichia coli* GM4 agreed reasonably with published values: a genome size of 3.95×10^6 bp and approximately two genomes per cell from a stationary batch culture in glucose minimal medium. *Desulfovibrio gigas* carried two plasmids of ~ 70 MDal (1.05×10^5 bp) and ~ 40 MDal (6×10^4 bp); *D. vulgaris* (Hildenborough) contained one of ~ 130 MDal (1.95×10^5 bp). Single plasmids were also detected in a second strain of *D. vulgaris* and in strain Berre sol of *D. desulfuricans* but not in 10 other desulfovibrios including representatives of *D. desulfuricans*, *D. vulgaris*, *D. salexigens* and *D. africanus*.

INTRODUCTION

Published information on the genomes of *Desulfovibrio* is restricted to the G + C ratios of various species (collated by Postgate, 1984) and the exploitation of DNA–rRNA hybridization by Pace & Campbell (1971) to illustrate inter-species relationships.

Yee & Inouye (1982) described a new electrophoretic procedure for sizing the prokaryotic genome. In principle, a digest of genomic DNA with a six-base recognition restriction enzyme is resolved by electrophoresis in agarose. The DNA in a strip of the agar is re-digested with a second such restriction enzyme and resolved by electrophoresis at right angles to the original current. Appropriate molecular weight markers are included at the second stage. Fragments are then sized with reference to the markers and summed to give the genome size. This procedure is complementary to, and consistent with, DNA reassociation curves, analyses of minimum DNA contents or sizing of electron micrographs (see Yee & Inouye, 1982).

We have applied this technique to single strains of *Desulfovibrio gigas* and *D. vulgaris*, in which we have also observed plasmids by conventional electrophoresis; we have also examined 12 other strains of *Desulfovibrio* for plasmids and observed them in two.

METHODS

Desulfovibrio gigas (NCIMB 9332), kindly provided by Dr E. C. Hatchikian, (CNRS, Marseille, France), was grown in a simple wier-type chemostat (V_F 540 ml) at $D = 0.025$ or 0.03 h^{-1} under O_2 -free N_2 at 28°C in lactate–sulphate medium C (Postgate, 1984) modified so that the population was limited by the N supply by including only $0.03 \text{ g NH}_4\text{Cl l}^{-1}$. Periodic cultural and visual checks were made for contamination (Postgate, 1984); none was present in any of the experiments reported here. *Desulfovibrio vulgaris* Hildenborough (NCIMB 8303) was grown in batch cultures (20 or 200 ml) to stationary phase under N_2 and was taken as N-limited. *Desulfovibrio gigas* and some other desulfovibrios (Table 2) were grown in normal medium C with extra NaCl as necessary. *Escherichia coli*

GM4 (a *pro leu thi lacY recA rpsL endA* derivative of K12) was grown in flasks in air at 28 °C shaken in the minimal glucose medium of Davis & Mingioli (1950) containing proline, leucine (each at 30 µg ml⁻¹) and thiamin (1 µg ml⁻¹).

DNA was extracted from *D. gigas* by the method of Marmur (1960) and tested with a variety of restriction enzymes: *EcoRI*, *BamHI*, *BstEII*, *SmaI*, *XhoI*, *HindIII*, *SalI* (BRL). Digestions were performed using the reaction buffer prescribed for each enzyme by the manufacturer. The first four gave good digests according to one-dimensional gels; the last three gave poor digests with only few fragments. Comparable tests were performed on DNA from *D. vulgaris* Hildenborough (*XhoI* gave satisfactory digests with this material), and the genomes of the two desulfovibrios were sized as described by Yee & Inouye (1982) using restriction enzymes which had given good digests. Each strain was examined twice, using different pairs of restriction enzymes. *Desulfovibrio gigas* DNA was digested with *EcoRI* or *BamHI* and, after electrophoresis, was re-digested *in situ* with *BstEII*. *Desulfovibrio vulgaris* DNA was digested with *EcoRI* or *XhoI* followed by *BstEII*. The standard marker was a *HindIII* digest of phage λ DNA (Boehringer-Mannheim). The procedure used with *E. coli* DNA was essentially similar (Robson *et al.*, 1984). For the gels shown in Fig. 1, genomic DNA (~20 µg) was digested for 120 min at 37 °C with 40 units *EcoRI* and one half of the digest was resolved by electrophoresis (22 h at 1.4 V cm⁻¹) in 0.7% (w/v) agarose gel. A strip of digested DNA was cut from the gel for re-digestion and the remainder was stained with ethidium bromide and inspected to identify the portion of the strip which carried the digested DNA. That portion was soaked in 'E' buffer [sodium acetate (20 mM), Tris/acetate (40 mM), Na₂EDTA (2 mM) pH 8.0] and transferred to a perspex container (Robson *et al.*, 1984). It was soaked overnight at 4 °C in reaction buffer containing 90 units *BstEII* and then re-digested for 6 h at 60 °C. The strip was removed, washed in fivefold-diluted buffer and applied to the edge of a second gel together with marker DNA. After electrophoresis the gel was stained with ethidium bromide, photographed and the print enlarged for sizing.

Plasmids were extracted as described by Cannon (1980). Samples were applied to 0.7% agarose in Cannon's TAE Buffer, resolved over 16 h at 4.4 V cm⁻¹, 80 mA, and photographed under 254 nm light after staining with 1 µg ethidium bromide ml⁻¹. A plasmid preparation from *Azotobacter chroococcum* strain MCC-1, which carries five cryptic plasmids (Robson *et al.*, 1984) served as standard for approximate molecular weight assessments; the smallest plasmid was not normally visible in our gels. Strains recorded as displaying no plasmids were checked three times in all.

Protein was measured by the Lowry method with BSA fraction 5 (Sigma; 1 mg ml⁻¹) as standard, checked to an absorbance of 0.667 at 279 nm. DNA was measured by the diphenylamine method of Burton (1956) using 80 ml culture samples concentrated ~20-fold. A freeze-thawed solution of herring sperm DNA (Boehringer-Mannheim) was used as standard. Bacterial dry weights were obtained by centrifuging triplicate 20–40 ml samples of culture (absorbance 0.36 to 0.4 at 540 nm), resuspending in 1 ml lactate-free medium C, measuring the volume and drying down 1 ml to constant weight at 80 °C compared with 1 ml suspending medium. Bacterial numbers were estimated in a Thoma bacterial counting chamber (20 µm depth), diluting the population and heating it briefly to 80 °C to arrest motility if necessary; the systematic error of Norris & Powell (1961) was disregarded. Absorbance values were read at 540 nm in a 1 cm cell in an EEL spectrophotometer and converted to dry weights.

RESULTS

Table 1 gives the analytical data for *D. gigas*, *D. vulgaris* and *E. coli* cultures grown at 28 °C. Protein contents of the organisms were relatively low, consistent with the N-limited status of the populations analysed. The substantial difference in total count between the two species, for a modest difference in absorbance, reflects the relatively large size of *D. gigas* (see Postgate, 1984). The mean DNA content per cell was 9.25×10^{-15} g for *D. vulgaris* and 17.7×10^{-15} g for the chemostat populations of *D. gigas*. Figure 1 (*a, b, c*) shows double-digest gels (*EcoRI* followed by

Table 1. *Analytical data for Desulfovibrio species and E. coli*

Organism	Population (cells ml ⁻¹)	Dry wt (µg ml ⁻¹)	$A_{540}^{1\text{cm}}$	DNA		Protein	
				(µg ml ⁻¹)	(% dry wt)	(µg ml ⁻¹)	(% dry wt)
<i>D. gigas</i>	3.04×10^8	343	0.38	4.75	1.38	215	62
	3.17×10^8	350	0.39	5.05	1.44	180	51
	4.5×10^7 *	99	0.14	1.4	1.42	—	—
<i>D. vulgaris</i>	6.97×10^8	200	0.31	5.75	2.8	100	50
<i>E. coli</i>	7.3×10^8	297	0.37	6.4	2.15	—	63†

* From batch culture in medium C, see text.

† From a second experiment.

Fig. 1. Two-dimensional double restriction digests of (a) *D. gigas*, (b) *D. vulgaris* Hildenborough, and (c) *E. coli* GM4. Marker DNA (*Hind*III fragments of phage λ) was run at the extremities. (d) Demonstration of plasmids in certain strains of *Desulfovibrio*; lane 1, *A. chroococcum* MCC-1 as control with plasmids of 190, 90, 45 and 40 MDal visible as well as smear of chromosomal DNA; lane 2 is irrelevant. Lane 3, *D. gigas*; 4, *D. desulfuricans* Berre sol; 5, *D. vulgaris* Hildenborough; 6, *D. vulgaris* Wandle; 7, *D. africanus* Walvis bay; 8, *D. vulgaris* Groningen; 9, *D. salexigens* California 43:63. For further details of strains see Table 2.

*Bst*EII) for *D. gigas*, *D. vulgaris* and *E. coli*. Although *D. gigas* and *D. vulgaris* have similar DNA base compositions at $\sim 65\%$ G + C (see Postgate, 1984), the patterns of the double digests show no resemblance, consistent with their being different species; comparative gels of two strains of *E. coli*, run in this laboratory, were almost identical. The number of fragments and pattern of the *E. coli* DNA gel immediately suggest that it has a larger genome than *Desulfovibrio*. Sizing and enumeration of the spots with subjective allowance for overlap gave mean genome sizes of $1.63 \pm 0.02 \times 10^6$ bp for *D. gigas* and $1.72 \pm 0.02 \times 10^6$ bp for *D. vulgaris* with a variance of 8%, corresponding to an average of 9.1 genomes per cell of *D. gigas* and 4.1 genomes per cell of *D. vulgaris*. *Escherichia coli* GM4 had a genome size of 3.94×10^6 bp, corresponding reasonably to published sizes collated by Yee & Inouye (1982); a similar calculation using the data in Fig. 1 indicated 2.1 genomes per cell.

The high genome complement of *D. gigas* prompted us to examine non-N-limited populations actively growing in batch culture in medium C. The population in Table 1 had the normal percentage of DNA per unit dry weight but possessed an average of 17.2 genomes per cell.

Figure 1(d) depicts one-dimensional gels of covalently closed circular plasmid DNA extracted from a variety of strains of *Desulfovibrio*. *Desulfovibrio gigas* possessed two plasmids of approximately 70 and 40 MDal; *D. vulgaris* strain Hildenborough had a plasmid of about

Table 2. *List of strains of Desulfovibrio tested for plasmids*

For further details of strains see Postgate (1984).

Species	Strain	NCIMB no.	Habit*	Plasmids (MDal)
<i>D. gigas</i>	—	9332	f	40, 70
<i>D. desulfuricans</i>	Berre sol	8388	f	90
	Norway 4	8310	s	0
	Canet 41	8393	s	0
	Teddington R	8312	f	0
	El Agheila Z	8380	s	0
<i>D. vulgaris</i>	Hildenborough	8303	f	130
	Wandle	8306	f	80
	Groningen	11779	f	0
<i>D. vulgaris</i> subsp. <i>oxamicus</i>	Monticello 2	9442	f	0
<i>D. africanus</i>	Benghazi	8401	f	0
	Walvis Bay	8397	s	0
<i>D. salexigens</i>	British Guiana	8403	s	0
	California 43:63	8364	s	0

* Fresh water (f) or saline (s) habit; 2.5% NaCl was added to medium C for the latter.

130 MDal, and single plasmids of about 80 and 90 MDal can be seen in *D. vulgaris* strain Wandle and *D. desulfuricans* strain Berre sol. None was found in the 10 other strains listed in Table 2. The two plasmids of *D. gigas* were stable in that they were conserved over 90 d (70 doublings) in the chemostat as well as over seven subcultures in the sulphate-free fumarate medium of Miller & Wakerley (1966).

DISCUSSION

Rapidly growing unicellular prokaryotes initiate chromosome replication in phase with their doubling time so, since the rate of chromosome duplication may be slower than the rate of cell duplication, individuals may carry DNA equivalent to more than one chromosome (see, for example, Pritchard *et al.*, 1969). We attribute our finding of an average of about two chromosomes per cell in *E. coli* GM4 to this phenomenon. The presence of an average of 9 to 17 genomes per cell in *D. gigas* might reflect a high frequency of initiation of replication combined with a remarkably low rate of DNA synthesis, but it is more likely to reflect multiple genomes in this organism. High chromosome copy numbers are known among unicellular prokaryotes. For example, DNA reassociation curves show that *Azotobacter vinelandii* is a multi-genomic unicellular prokaryote which normally possesses an average of 20 to 40 genomes per cell (Sadoff *et al.*, 1979), a finding confirmed in principle in our laboratory for *A. chroococcum* (Robson *et al.*, 1984). Similar studies with *Micrococcus radiodurans* (Hansen, 1978) indicated four to eight genomes per cell depending on the growth rate, a character which may be relevant to its radiation resistance and which was confirmed by studies of transformation frequencies (Tirgari & Moseley, 1980). Multiple chromosomes were implied by the study by Mann & Carr (1974) of the unicellular cyanobacterium *Anacystis nidulans*. Although *D. gigas* appears also to be a multinucleate prokaryote, the status of *D. vulgaris*, with an average of four genomes per cell, is on the borderline and studies of the effect of growth rate would be needed to establish whether it ever reaches a mean copy number of one chromosome per cell.

The genomes of the two desulfovibrios studied are completely different but both are small: around 1.1×10^9 Dal compared with 2.56×10^9 Dal for *E. coli* in our hands and 3.793×10^9 Dal for *Myxococcus xanthus* (Yee & Inouye, 1982); they are comparable in size to the genomes of certain Brucellaceae or *Neisseria catarrhalis* (Bak *et al.*, 1970). The small genome may reflect the rather limited metabolic capabilities of these species and their strictly anaerobic habit (see Postgate, 1984).

The genus *Desulfovibrio* now joins the many groups of prokaryotes in which cryptic plasmids are found but, if our limited survey is any guide, they show no consistency as regards size, multiplicity or incidence among these bacteria. Useful discussion of their roles must await the preparation of cured strains or appropriate DNA probes. Our data provide little information concerning their copy numbers. We have seen no particularly intense bands in restriction digests such as occur when there is a high plasmid copy number relative to the chromosome. Their stability requires an average of only one per cell, yet they could equal or even exceed by a small margin the chromosomal copy numbers. If there is just one copy of each plasmid per chromosome, then over 10% of the genome of *D. gigas* or *D. vulgaris* Hildenborough is plasmid DNA.

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