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Genome Analysis of Several Marine, Magnetotactic Bacterial Strains by Pulsed-Field Gel Electrophoresis

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Abstract. The genomic DNA of three strains of marine magnetotactic bacteria, including two facultatively anaerobic vibrios, strains MV-1 and MV-2, and the microaerophilic coccus, strain MC-1, was analyzed by pulsed-field gel electrophoresis (PFGE). Digestion of the genomic DNA of strain MV-1 by the restriction endonucleases *Avr*II, *Bam*HI, *Hin*dIII, *Nhe*I, *Sal*I, *Sfi*I, *Sgf*I, *Sgr*AI, and *Xba*I resulted in a large number of fragments below 400 kb that were difficult to resolve by PFGE. Digestion of MV-1 DNA with *Not*I and *Rsr*II resulted in no fragments. Treatment of genomic DNA of strains MV-1 and MV-2 with *Pac*I, *Pme*I, and *Spe*I yielded a manageable number of fragments (ca. 20) that were relatively easily resolved with PFGE, while *Pac*I and *Spe*I were effective for strain MC-1. There was no evidence for the presence of plasmids and linear chromosomes in any of the strains, and strains MV-1 and MV-2 appear to contain a single, circular chromosome. Genome sizes of strains MV-1, MV-2, and MC-1 were estimated to be between 3.6 and 3.9 Mb (mean ± SD; 3.7 ± 0.2), 3.3 and 3.7 Mb (3.6 ± 0.2), and 4.3 and 4.7 Mb (4.5 ± 0.3), respectively. The restriction fragment patterns of the vibrioid strains MV-1 and MV-2 were extremely similar, suggesting that the strains are closely related.

Magnetotactic bacteria are a morphologically and metabolically diverse group of Gram-negative prokaryotes that passively align and actively swim along the Earth's geomagnetic field lines [5, 12]. This feature, termed magnetotaxis [11], results from the presence of unique intracellular structures called magnetosomes [1] that are membrane-bound crystals of magnetite (Fe_3O_4) [20] or greigite (Fe_3S_4) [24]. Although most magnetotactic bacteria produce species- or strain-specific crystal morphologies of only one mineral [2], some species contain both minerals [6, 8]. The apparent function of magnetotaxis is to aid chemotactic cells to more efficiently locate and maintain an optimal position in vertical chemical gradients (e.g., a preferred oxygen concentration) common in many aquatic habitats [21].

The described magnetotactic bacterial strains are obligate microaerophiles [3, 20], obligate anaerobes [32], or facultatively anaerobic microaerophiles [5, 25] and are difficult to cultivate. Because of their fastidiousness, effective genetic systems have not been established for most of the magnetotactic bacterial strains in pure culture. Thus, little is known about how these organisms synthesize magnetosomes at the molecular level. While we are taking several approaches to understanding how magnetotactic bacteria form magnetosomes, we believe some valuable genetic information could be obtained by determining the genome size and organization of different magnetotactic bacterial strains.

The purpose of this study was to estimate the genomic size and organization of strains of magnetotactic bacteria by PFGE. The development of this technique has made it possible to separate large DNA fragments [34] whose molecular weights can be determined and used to estimate genome sizes. During the past decade, PFGE has been used in many molecular techniques, including bacterial strain typing and identification, genome mapping, electrophoretic karyotyping, isolation of large pieces of DNA, and separation of different topological forms of DNA [26]. The results presented here are the first descriptions of magnetotactic bacterial genomes and represent the initial steps in the construction of physical maps of the genomes of magnetotactic bacteria.

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Materials and Methods

Organisms and growth conditions. The marine magnetotactic bacterial strains used in this study included two vibrioid-to-helical organisms, designated MV-1 and MV-2, and one marine coccoid strain designated MC-1. Strain MV-1 was isolated from a salt marsh pool in Neponset River Estuary (Boston, Mass., USA) [5], and strains MV-2 and MC-1 were isolated from the Pettaquamscutt Estuary (Narragansett Bay, R.I., USA) [27, 28].

Cells of strain MV-1 were grown anaerobically under 1 atm of nitrous oxide (N_2O) as the terminal electron acceptor, in a diluted artificial sea water [7] containing (per liter): 0.2 ml 0.2% aqueous resazurin; 5.0 ml modified Wolfe's mineral elixir [21]; 0.5 g sodium succinate · $6H_2O$; 0.5 g CasAmino Acids (Difco Laboratories, Detroit, Mich., USA); 0.2 g sodium acetate · $3H_2O$; 0.25 g NH_4Cl; 2.4 ml 0.8 M $NaHCO_3$; 1.5 ml 0.5 M $KHPO_4$ buffer, pH 7.1; 0.5 ml vitamin solution [21]; 2.0 ml of neutralized 0.43 M cysteine HCl · H_2O; and 2.5 ml 0.01 M ferric quinate solution [12]. The mineral elixir, resazurin, sodium succinate, CasAmino acids, sodium acetate, and NH_4Cl were added to the seawater, and the pH was adjusted to 7.0. The medium was then sparged with N_2 at approximately 100 ml min^{-1} for about 45 min and then with pure N_2O at the same flow rate for another 45 min and autoclaved. After the growth medium had cooled to room temperature, the $KHPO_4$ buffer, $NaHCO_3$, vitamin, cysteine, and ferric quinate solutions were added in order by syringe from sterile anaerobic stock solutions. All solutions were under 1 atm N_2 except the $NaHCO_3$, which was under 1 atm CO_2, and the cysteine solution, which was prepared fresh, filter-sterilized, and injected directly into the medium without gassing. The final pH was approximately 7.1, and the growth medium was inoculated after it turned from pink to colorless.

Strain MV-2 was grown in the same medium, except the sodium succinate concentration was increased to 1.0 g per liter and the CasAmino Acids were omitted. Cells of strain MC-1 were grown in the artificial seawater described above containing (per liter): 0.2 ml of 0.2% aqueous resazurin; 5.0 ml modified Wolfe's mineral elixir; 2.5 g $Na_2S_2O_3$ · $5H_2O$; 1.26 g $NaHCO_3$; 1.0 g NH_4Cl; 1.5 ml 0.5 M $KHPO_4$ buffer, pH 7.1; 0.5 ml vitamin solution; 2.0 ml 0.14 M neutralized cysteine HCl · H_2O; and 2.5 ml 0.01 M ferric quinate solution. All ingredients were added to the seawater except the vitamin, cysteine, and ferric quinate solutions, and the pH was adjusted to 7.0. The medium was then sparged with 7.5% CO_2 in 92.5% N_2 for 45 min at approximately 100 ml min^{-1} and autoclaved. The vitamin, cysteine, and ferric quinate solutions were added as described in the previous paragraph. After the growth medium became colorless, the bottle was inoculated, and sterile pure O_2 was injected into the bottle without shaking to a headspace concentration of 1%. As cells increased in number and consumed O_2, additional sterile O_2 was added to the culture in increments of 1–2% (vol/vol) of the headspace.

Preparation of genomic DNA. Approximately 2×10^9 cells, as determined by direct cell counts using a Petroff-Hausser bacterial counting chamber, were used for each ml of agar plugs. Cells were harvested by centrifugation at 10,000 *g* in a microcentrifuge for 3 min at 25°C, then resuspended in 0.5 ml cell suspension buffer (10 mM Tris-HCl, pH 7.2, 20 mM NaCl, 50 mM EDTA, pH 8.0). An equal amount of 1.5% InCert Agarose (FMC Bioproducts, Rockland, Me., USA) was added to the suspension at 50°C. This mixture was transferred to plug molds (Bio-Rad; Hercules, Calif., USA) and allowed to solidify at 4°C. After removal from the mold, plugs were incubated in lysozyme solution (10 mM Tris-HCl, pH 7.2, 50 mM NaCl, 0.2% sodium deoxycholate, 0.5% sodium lauryl sarcosine, and 1 mg ml^{-1} lysozyme) for 2 h at 37°C. Plugs were then treated overnight with proteinase K solution (100 mM EDTA, pH 8.0, 0.2% sodium deoxycholate, 1.0% sodium lauryl sarcosine, 1 mg ml^{-1} proteinase K) at 50°C and then washed five times with 1× wash buffer (20 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0), each wash for 1 h at 25°C with gentle agitation, followed by two additional washes in 0.1× wash buffer. Plugs were stored at 4°C in 0.1× wash buffer. When required, chromosomal DNA was linearized by causing randomly occurring breaks in the DNA as previously described [18].

Restriction enzyme digestion. Individual plugs contained ~0.3 µg genomic DNA and were incubated in 1× restriction enzyme buffer at 25°C for 30 min. The buffer was then replaced with 250 µl of fresh buffer and the appropriate amount of restriction endonuclease. Enzyme concentrations and digestion conditions were according to the manufacturer's suggestions (New England Biolabs, Beverly, Mass., USA). After incubation, plugs were washed with 1× wash buffer for 30 min at 25°C and allowed to equilibrate in 0.5× TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0) for 15 min. One-third of each plug was then loaded into a well of a 1.0% Pulsed-Field Certified Agarose (Bio-Rad) gel and sealed with warm agarose.

Electrophoresis conditions. Electrophoresis was performed with the CHEF-DR III System (Bio-Rad). Most gels were run at 14°C, 6 V/cm, with a 120° reorientation angle with 0.5× TBE buffer. Both the buffer and the gel were cooled to 14°C prior to the start of each run. Switch times and gel run times varied according to the size of fragments being resolved [10]. For the resolution of DNA fragments up to 2 Mb, gels were run for 24 h with a switch time of 90–120 s. Fragments from 300 to 800 kb were resolved in gels run for 22 h and switch times of 50–90 s. Mid-sized fragments ranging from 50 to 300 kb were separated in gels run for 22 h with a 5–40 (occasionally 5–20) s switch time. Smaller fragments (<50 kb) were separated on gels run for 16 h with a 1–5 s switch time.

Size determination of DNA fragments and estimation of genome size. Following electrophoresis, gels were stained with 0.1 µg $µl^{-1}$ ethidium bromide for 10 min and destained in distilled water for 30 min. Gels were photographed with a Gel Doc 1000 System (Bio-Rad). The sizes of fragments generated from restriction digestions were calculated with standard curves created by plotting the logarithm of the fragment molecular weight in kilobases versus distance traveled in mm from the wells. *Saccharomyces cerevisiae* YNN295 chromosomes, lambda DNA concatemers, and an 8- to 48-kb DNA size standard (all from Bio-Rad) were used as size markers. Molecular weights of all fragments produced by *Pac*I, *Pme*I, and *Spe*I digestions of genomic DNA of all three strains were calculated from an average of three to five pulsed-field gels. The total molecular weight of the genomes of each organism was determined by adding the sizes of the fragments produced by each restriction endonuclease.

Results

Identification of effective restriction endonucleases. The overall G + C mol % of the genomic DNA of the three strains of magnetotactic bacteria studied is within the range of 52–57% (D.A. Bazylinski, unpublished data) relatively close to that of *Escherichia coli* [29]. Therefore, restriction endonucleases that would cleave strain MV-1 genomic DNA into a reasonable number of fragments were first chosen based on the following criteria: those that were used previously to map the *E. coli* chromosome [16, 22, 30, 31, 35], those that recognized 8-bp sequences, and those that included sequences such as CTAG, that have been reported to be rare in prokary-

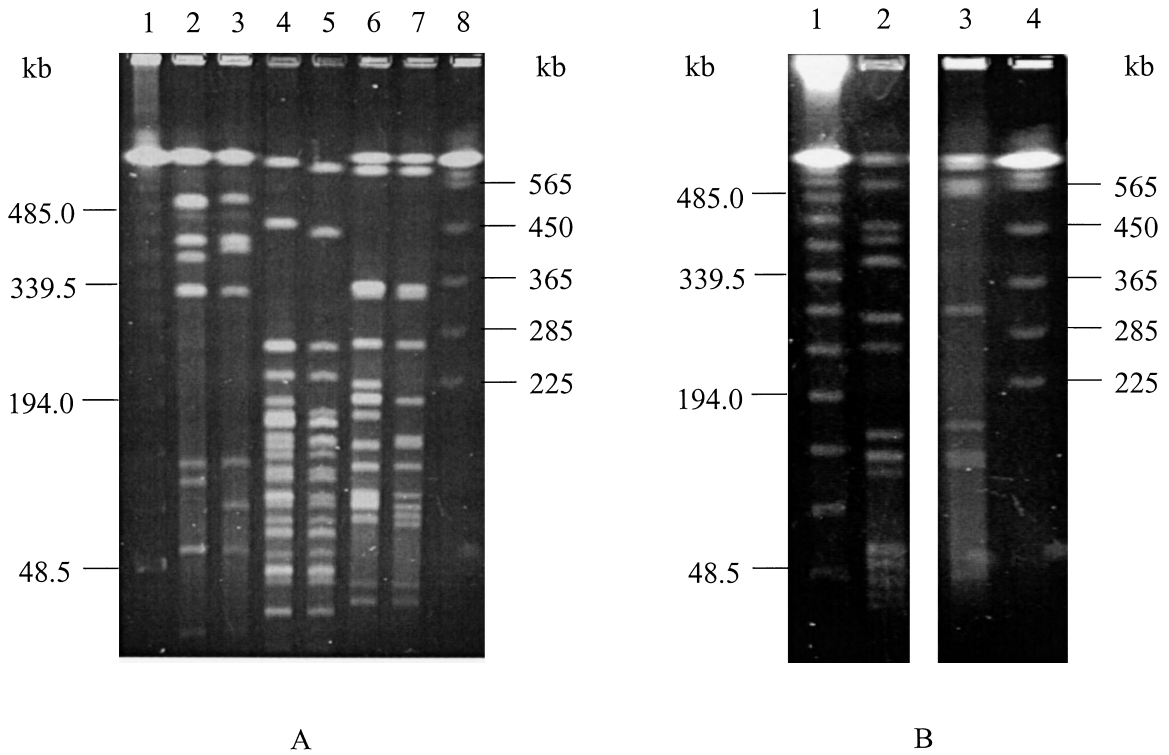

Fig. 1. PFGE of genomic DNA from magnetotactic bacterial strains MV-1, MV-2, and MC-1. (A) vibrioid strains, MV-1 and MV-2; lanes 1 and 8, DNA size standards with molecular weights in kb; lanes 2 and 3, MV-1 and MV-2 DNA, respectively, digested with *Pac*I; lanes 4 and 5, MV-1 and MV-2 DNA, respectively, digested with *Pme*I; lanes 6 and 7, MV-1 and MV-2 DNA, respectively, digested with *Spe*I. (B) coccoid strain, MC-1; lanes 1 and 4, DNA size standards; lanes 2 and 3, MC-1 DNA digested with *Pac*I and *Spe*I, respectively. The electrophoresis conditions used here were optimized for mid-sized fragments ranging from ~50 to ~300 kb.

otes [26]. Digestion with *Avr*II, *Bam*HI, *Hin*dIII, *Nhe*I, *Sal*I, *Sfi*I, *Sgf*I, *Sgr*AI, and *Xba*I resulted in a very large number of fragments below 400 kb, that were difficult, if not impossible, to resolve by PFGE. Digestion with *Not*I and *Rsr*II resulted in no fragments. The intron-encoded endonuclease I-*Ceu*I was also tested, but the resulting DNA fragment patterns were inconsistent and deemed unreliable for genome size estimation. Restriction endonucleases that generated a manageable number of fragments that were relatively easily resolvable by PFGE were *Pac*I (TTAAT′TAA), *Pme*I (GTTT′AAAC), and *Spe*I (A′CTAGT). The same three enzymes were used to analyze the MV-2 genome, while *Pac*I and *Spe*I were effective and used for MC-1 genome analysis. Figure 1A depicts a typical example of a pulsed-field gel showing resolution of mid-sized fragments produced by the digestion of genomic DNA of the vibrioid strains with *Pac*I, *Pme*I, and *Spe*I. Figure 1B depicts similarly sized fragments produced by digestion of MC-1 genomic DNA with *Pac*I and *Spe*I.

Analysis of MV-1 and MV-2 genomes. The restriction fragment patterns of the genomic DNA of strains MV-1 and MV-2 are shown in Table 1. Of the nine fragments obtained by *Pac*I digestion of MV-1 DNA, six virtually identically sized fragments were produced from MV-2 DNA. Unique fragments were observed at approximately 375, 124, and 110 kb for strain MV-1, and approximately 379, 123, and 92 kb for strain MV-2. Of the 23 fragments produced by *Pme*I digestion of MV-1 DNA, 17 similarly sized fragments were found in MV-2. Unique fragments were seen at approximately 650, 431, 195, 177, 154, and 136 kb for MV-1 and at approximately 578, 426, 187, 150, and 133 kb for MV-2. Of the 17 fragments produced by *Spe*I digestion of MV-1 DNA, 12 were also found in MV-2 DNA. Unique fragments for MV-1 were found at 200, 167, 128, 98, and 94 kb, and for MV-2 at approximately 147, 129, 96, and 89 kb. Addition of the fragments resulting from MV-1 DNA treatment with *Pac*I, *Pme*I, and *Spe*I resulted in genome size estimations of 3.7, 3.6, and 3.9 Mb, respectively, giving an average of 3.7 ± 0.2 Mb (Table 1). Treatment with the same three enzymes on MV-2 genomic DNA resulted in genome size estimations of 3.7, 3.3, and 3.7 Mb, respectively, resulting in an average genome size of 3.6 ± 0.2 Mb (Table 1).

Genome analysis of MC-1. Digestion of MC-1 genomic DNA with the restriction endonucleases *Pac*I and *Spe*I

Table 1. DNA restriction fragments (kb) generated by *Pac*I, *Pme*I, and *Spe*I digestions of genomic DNA and estimated genome sizes of strains MV-1 and MV-2. Fragment sizes unique to each strain are in bold

	*Pac*I		*Pme*I		*Spe*I	
Fragment	MV-1	MV-2	MV-1	MV-2	MV-1	MV-2
A	1129	1129	**650**		1114	1114
B	675	667		**578**	609	609
C	497	493	**431**		327	327
D	403	393		**426**	323	323
E		**379**	264	267	251	254
F	**375**		227	230	**200**	
G	312	309	**195**		186	186
H	**124**			**187**	**167**	
I		**123**	**177**			**147**
J	**110**		170	173	147	144
K		**92**	170	173		**129**
L	64	65	**154**		**128**	
M				**150**	100	103
N			146	145	**98**	
O				**133**		**96**
P			**136**		**94**	
Q			120	117		**89**
R			115	111	86	86
S			95	95	37	37
T			90	90	25	25
U			85	76	24	24
V			76	70		
W			74	68		
X			60	58		
Y			51	50		
Z			47	47		
AA			39	39		
BB			19	20		
Total	3690	3651	3589	3303	3919	3695

Table 2. DNA restriction fragments (kb) generated by *Pac*I and *Spe*I digestions of genomic DNA and estimated genome size of strain MC-1

	MC-1	
Fragment	*Pac*I	*Spe*I
A	774	862
B	588	838
C	469	652
D	422	551
E	405	535
F	366	491
G	314	312
H	248	167
I	155	141
J	129	131
K	119	43
L	76	20
M	83	
N	72	
O	56	
Total	4275	4743

resulted in 15 and 12 fragments, respectively, and restriction fragment patterns very different from those of MV-1 or MV-2 with the same endonucleases (Table 2, Fig. 1). Addition of the DNA fragments resulting from the digestion by *Pac*I and *Spe*I shows the genome size of strain MC-1 to be 4.3 and 4.7 Mb, respectively, resulting in an average genome size of 4.5 ± 0.3 Mb (Table 2).

Chromosomal organization. Unrestricted genomic DNA from all strains did not migrate into the gel from the wells in which it was loaded during PFGE under typical conditions (Fig. 2A). The presence of linear chromosomal DNA was tested for by electrophoresis of unrestricted DNA at 2 V/cm in 0.8% agarose in 1× TAE with an included angle of 106° and a 30-min switch time for 72 h. Under these conditions, linear fragments of DNA ranging from 1.6 Mb to 5.7 Mb, molecular weight markers of the yeast *Schizosaccharomyces pombe*, could be easily resolved. Faint, indistinct bands of high molecular weight DNA were observed at approximately 3.5 Mb in the lanes containing undigested genomic DNA of the strains MV-1 and MV-2 under these conditions. No other bands were observed in the 1.6 to 5.7 Mb size range in these gels. These faint bands probably represent circular genomic DNA that had been linearized during preparation of the DNA plugs. Bands were never observed for strain MC-1 under any conditions during PFGE of unrestricted genomic DNA. Deliberate attempts at linearizing circular chromosomal genomic DNA, as previously described [18], to make the bands of chromosomal DNA more intense were not successful for any of the strains, nor did they result in the observation of bands during PFGE of unrestricted genomic DNA from strain MC-1.

Uncut, undigested genomic DNA from all strains was electrophoresed alongside that treated with a restriction endonuclease under every PFGE DNA size resolving condition. There was no evidence of extrachromosomal DNA (e.g., plasmids) with a molecular weight of between, we estimate, about 5.7 Mb and about 1 kb in any of the strains. Figure 2A depicts both unrestricted and *Spe*I-treated genomic DNA from all three strains with resolution of fragments in the 300 kb to 2 Mb size range and shows the absence of distinct bands in lanes containing undigested genomic DNA. Fragments near the bottom of the gel (bracketed area) in Fig. 2A were more easily resolved in the gel shown in Fig. 2B, which depicts unrestricted and *Spe*I-treated genomic DNA in the 50- to 300-kb size range, and again, distinct bands of DNA are absent in lanes containing untreated genomic DNA in this size range. In this gel, the bands observed above 365 kb in lanes containing untreated and restriction endonuclease-treated genomic DNA and molecular weight markers

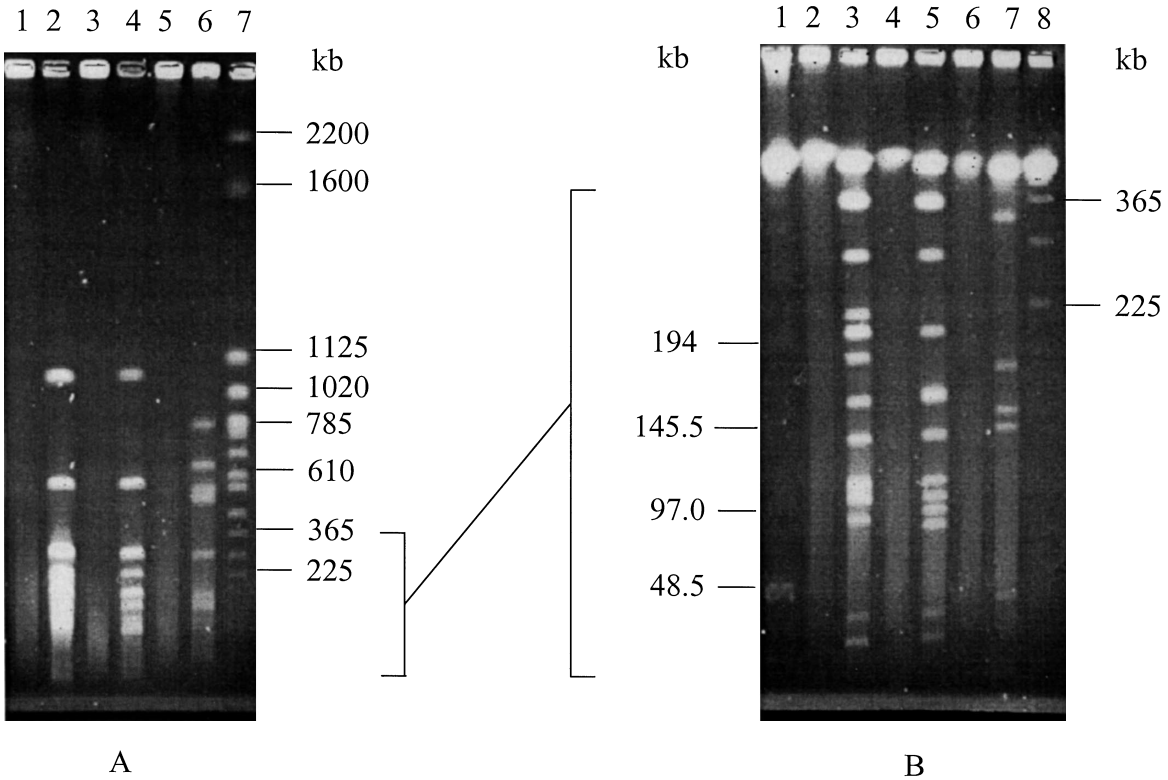

Fig. 2. PFGE of untreated and *Spe*I-treated genomic DNA from magnetotactic bacterial strains MV-1, MV-2 and MC-1. (A) Gel in which PFGE conditions were optimized for the resolution of DNA fragments ranging up to 2 Mb. Lanes: 1 and 2, untreated and *Spe*I-treated genomic DNA, respectively, from strain MV-1; 3 and 4, untreated and *Spe*I-treated genomic DNA, respectively, from strain MV-2; 5 and 6, untreated and *Spe*I-treated genomic DNA, respectively, from strain MC-1; lane 7, DNA size standard with molecular weights in kb. (B) Gel in which PFGE conditions were optimized for the resolution of DNA fragments ranging from ~50 to ~300 kb, those which were not easily resolved in gel A. Lanes 1 and 8, DNA size standards; lanes 2–7 contain untreated and *Spe*I-treated genomic DNA, respectively, from strain MV-1 (lanes 2 and 3), strain MV-2 (lanes 4 and 5), and strain MC-1 (lanes 6 and 7). Bracketed area in gel A represents bracketed area in gel B based on DNA fragments present in the bracketed areas. The intensely staining band in lanes containing unrestricted genomic DNA in B, just above the 365-kb marker, is probably an accumulation of sheared or randomly broken genomic DNA fragments larger than 365 kb that migrate to this area on the gel (see text). The similar band in the other lanes contains unresolvable DNA fragments larger than 365 kb along with any large pieces of sheared or broken DNA. Note that this band is absent in A.

probably represent an accumulation of sheared or randomly broken genomic DNA fragments above 365 kb that migrated to this area on the gel. These bands were not seen in the lanes containing untreated genomic DNA in the gel shown in Fig. 2A, where fragments of this size would be more easily resolved. Likewise, gels showing resolution of smaller fragments ranging in size from 8 to 100 kb also did not reveal the presence of distinct bands in lanes containing untreated genomic DNA in this size range (data not shown).

Discussion

The use of PFGE in this study has revealed some significant and valuable information on the genomic organization and genome size of some magnetotactic bacteria. First, our results indicate that the magnetotactic bacterial strains, MV-1, MV-2, and MC-1, contain circular and not linear chromosomes. Linear DNA, even large molecules, will migrate into an agarose gel during PFGE, unlike large circular molecules, which remain trapped in the well [13, 14, 18]. No distinct bands were observed during PFGE on unrestricted genomic DNA from any of the strains, indicating the absence of linear DNA. Very large circular DNA molecules can become randomly linearized during preparation of the genomic DNA and enter the gel [13, 18, 23]. This observation has revealed the presence of multiple chromosomes or large replicons in certain organisms [18, 23]. We observed very faint high molecular weight bands in lanes containing unrestricted genomic DNA from strains MV-1 and MV-2, probably resulting from linearization of the genomic DNA during cell lysis. Attempts at linearizing the chromosome under alkaline lysis conditions did not improve the appearance of the bands. Since these bands migrated at approximately 3.5 Mb for strains MV-1 and MV-2,

similar to the genome sizes estimated from addition of restriction fragments for these strains, it seems likely that the vibrioid strains contain a single circular chromosome. This result is interesting in that results from a recent study [23] show that a great deal of diversity regarding genome size and organization exists in the α-subgroup of the class *Proteobacteria*, the subgroup that includes virtually all the magnetite-producing magnetotactic bacteria including strains MV-1, MV-2, and MC-1 [8, 17]. Members of the α-subgroup of the *Proteobacteria* not only show great differences in genome size, but many possess megabase-sized replicons, linear chromosomes, and multiple chromosomes [23]. The fact that bands were not detected during PFGE of unrestricted genomic DNA from strain MC-1 suggests that this strain also contains circular chromosomal DNA, although we are unable to conclude that there is a singular chromosome.

Typical PFGE conditions as used in this study would likely reveal any extrachromosomal DNA, such as plasmids, during PFGE of unrestricted genomic DNA [23]. We did not find any evidence of extrachomosomal DNA in the form of discrete bands of DNA in any of the strains in PFGE gels of unrestricted genomic DNA under any condition used to resolve different sizes of DNA fragments. We were also unable to recover any plasmid DNA, using various well-established methods [33]. Because the trait of magnetosome synthesis is found in many disparate groups of bacteria [4], the possibility of the genes for magnetosome synthesis being located on a plasmid must be seriously considered. To date, plasmids have never been observed or recovered from any magnetotactic bacterial strain. However, our results do not completely eliminate the possibility that strains MV-1, MV-2, and MC-1 carry plasmids that cannot, for some reason, be visualized by PFGE.

Bacterial genomes range in size from 600 kb to 9.5 Mb [15]. The genome sizes of the magnetotactic strains used in this study are 3.7, 3.6, and 4.5 Mb for strains MV-1, MV-2, and MC-1 respectively. According to the classification of Cole and Saint Girons [15], these magnetotactic bacterial strains would fall into their Group 3, prokaryotes that have intermediate-sized genomes ranging from 3 to 4.5 Mb.

Although strains MV-1 and MV-2 were isolated from different sources, phylogenetic analyses of strains MV-1 and MV-2 show that the small subunit rRNA sequences of the strains are essentially identical [17], indicating a close evolutionary and genetic relatedness between the strains. However, Fox et al. [19] have determined that identity of 16S rRNA sequences is not necessarily a sufficient criterion to guarantee species identity. We show here that the restriction fragment patterns of the genomic DNA and the genome size estimation of the two vibrioid strains using the same restriction endonucleases are similar but not identical, again indicating that a relatively close genetic relationship exists between the strains. Given this observation, the fact that genome sizes vary greatly within strains of the same species, even as much as 1 Mb in certain isolates of *Escherichia coli* [9], and the remarkable similarity in their phenotypic traits (e.g., modes of metabolism; D.A. Bazylinski, unpublished data), it seems likely that MV-1 and MV-2 represent different strains of the same species. Strain MC-1, on the other hand, shows no evidence of genetic relatedness to the vibrioid strains on the basis of PFGE of genomic DNA, as might be expected.

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