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Physical and genetic characterization of the genome of *Magnetospirillum magnetotacticum*, strain MS-1

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Abstract

Pulsed-field gel analysis of *Magnetospirillum magnetotacticum,* strain MS-1, indicates that the genome is a single, circular structure of about 4.3 mb. A few genes, identified by sequence similarity, have been localized and arranged in a map with *dnaA*, indicating the presumed origin of replication. There are at least two rRNA operons. In addition, rRNA genes are found on a 40 kb, possibly extrachromosomal, structure. The genes thought to be involved in magnetite synthesis, *bfr* and *magA*, are located in the same 17% of the genome. A one base pair-overlap seen in the *bfr* genes of MS-1 is found also in the closely related magnetic strain AMB-1, but not in the non-magnetic relative *A. itersonii.*

Keywords: Bacterioferritin; Genetic map; Magnetite; Pulsed-field gels; Sequences

1. Introduction

Magnetospirillum magnetotacticum, strain MS-1, first isolated by Blakemore in 1975, converts iron into single-domain crystals of the iron mineral magnetite. The crystals remain within the organism, organized in long chains that retain their north/south orientation. As a result, MS-1 is sensitive to the earth's magnetic field and is attracted to a magnet placed on the wall of a culture vessel.

Like most magnetite-producing strains so far described, MS-1 has been classified as belonging to the α-subgroup of the proteobacteria. Some members of this group are photosynthetic. In these cases, most of the genes involved in photosynthesis are found to be clustered in superoperons, occupying about 1% of the genome. This observation suggested the possibility that the genes involved in magnetite synthesis might be similarly clustered. The potential for clustering, together with a need for information about physical structure, made a characterization of the genome with respect to size, structure and gene distribution desirable. Some members of the α-proteobacteria have been physically characterized (Jumas-Bilak et al., 1998) and found to have unusual genome structure, including linear and multiple chromosomes. A more recent study of some marine magnetotactic strains (Dean and Bazylinski, 1999), however, found no unusual structures. The genome sizes of these organisms were given as approximately 3.6, 3.7 and 4.5 mb, but there was no genetic characterization.

We have examined the situation in MS-1 by constructing a low-resolution physical map of the genome using pulsed-field gel electrophoresis (PFGE). The size of the genome has been determined as well as its structure, i.e. the number of chromosomes, whether they are linear or circular, and if there are any plasmids present. By probing Southern blots of the gels with cloned DNA from MS-1, it is possible to arrange the restriction enzyme fragments on a single, circular structure and localize on it the few MS-1 genes so far

Abbreviations: aa, amino acid(s); Ad, *Aquaspirillum dispar*; Ai, *Aquaspirillum itersonii*; AMB-1, *Magnetospirillum* sp. strain AMB-1; *bfr1*and *bfr2,*genes coding for bacterioferritin subunits; bp, base pair(s); Ec, *Escherichia coli*; kb, kilobase(s) or 1000 bp; *magA,*gene coding for the MagA protein; mb, megabase(s) or 1,000,000 bp; MS-1, *Magnetospirillum magnetotacticum,*strain MS-1; ORF, open reading frame; Pc, fragments produced by the restriction enzyme, *PacI*; PFGE, pulsed-field gel electrophoresis; Pm, fragments produced by the restriction enzyme, *PmeI*; PmPc, fragments resulting from double digestion with restriction enzymes *PmeI*and *PacI*; rRNA, ribosomal RNA; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Sw, fragments produced by the restriction enzyme, *SwaI*; SwPm, fragments resulting from double digestion with restriction enzymes *SwaI* and *PmeI*

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available. These genes include three that might be involved in magnetite synthesis, *bfr*1, *bfr*2 and *mag*A. The *bfr* genes are known to be tightly linked and encode subunits of the iron storage protein, bacterioferritin (Bertani et al., 1997), which may be the source of the iron that is converted to magnetite. The *mag*A gene, first isolated in *Magnetospirillum* sp. strain AMB-1 (AMB-1; Matsunaga et al., 1991, 1997), produces a component of the magnetosomal membrane that surrounds each magnetite crystal. We have isolated the homologous *mag*A gene from strain MS-1 for this study.

2. Materials and methods

2.1. Bacterial strains

Strains MS-1 (ATCC 31632) and AMB-1 (ATCC 700264), as well as Ad (ATCC 27510) and Ai (ATCC 12639), were purchased from the American Type Culture Collection (Rockville, MD) and grown according to their recommendations.

2.2. Preparation of plugs for PFGE

Cultures of strain MS-1 were harvested by centrifugation before reaching saturation. Approximately 2×10^9 bacteria were pelleted, washed once in 1 ml TE and resuspended in 1 ml 50 mM Tris, 100 mM EDTA, containing 25% sucrose. Two-hundred microlitres of 4 mg/ml lysozyme (Sigma, St. Louis, MO) and 1.8 ml of 1% InCert agarose (FMS BioProducts, Rockland, ME) containing 0.25 M EDTA were added and the mixture was poured into 1 cm^2 molds. Following solidification, the agarose was expelled from the mold and sliced with a razor blade into 1.5 mm thick plugs, which were then digested for two days at 37°C in 10 ml 1% sarkosyl, 0.2 mg/ml proteinase K (Boehringer–Mannheim, Indianapolis, IN), in 0.5 M EDTA. The plugs were then washed repeatedly with 50 mM EDTA and stored at 4°C.

2.3. Digestion of PFGE plugs with restriction enzymes

Restriction enzymes *Pac*I and *Pme*I were obtained from NE Biolabs, Beverly, MA; *Swa*I from Boehringer-Mannheim. Single plugs were equilibrated first with TE, then overnight at 4°C with the appropriate restriction enzyme buffer, and finally digested overnight with 40 units of enzyme in a volume of 100 μl.

2.4. Pulsed-field gel electrophoresis

One percent agarose gels were subjected to electrophoresis using a Chef-DRII System (Bio-Rad Laboratories, Hercules, CA) with lambda 50 kb ladder (Bio-Rad), Sc chromosomes (Bio-Rad), or Sp chromosomes (BioRad) as molecular weight markers. The parameters for each run are detailed in the figure legends.

2.5. Probing of Southern blots

Pulsed-field gels of digested MS-1 DNA were blotted onto nylon membranes (Hybond-N; Amersham, Arlington Heights, IL) using procedures described in Sambrook et al. (1989) and probed with specific MS-1 DNA sequences. The sequences were cloned from partial, small-insert libraries of MS-1 DNA, produced by digesting MS-1 DNA with either of the restriction enzymes *Eco*RI or *Hind*III (NE Biolabs, Beverly, MA), ligating the fragments into the Bluescript II KS ± vector (Stratagene, La Jolla, CA), and transforming the resultant plasmids into XL-1 bacteria (Stratagene). The clones were digested with suitable enzymes and insert DNA separated from the vector by electrophoresis. Insert DNA was extracted from the gel, using a Qiagen Gel Extraction Kit (Qiagen, Chatsworth, CA), labeled with α^{32}P-dCTP (Amersham), using a Random Primed DNA Labeling Kit (Boehringer Corp., Indianapolis, IN), and used to probe the pulsed-field gel blots, using hybridization procedures recommended by Hybond.

2.6. Sequencing and identification of genes

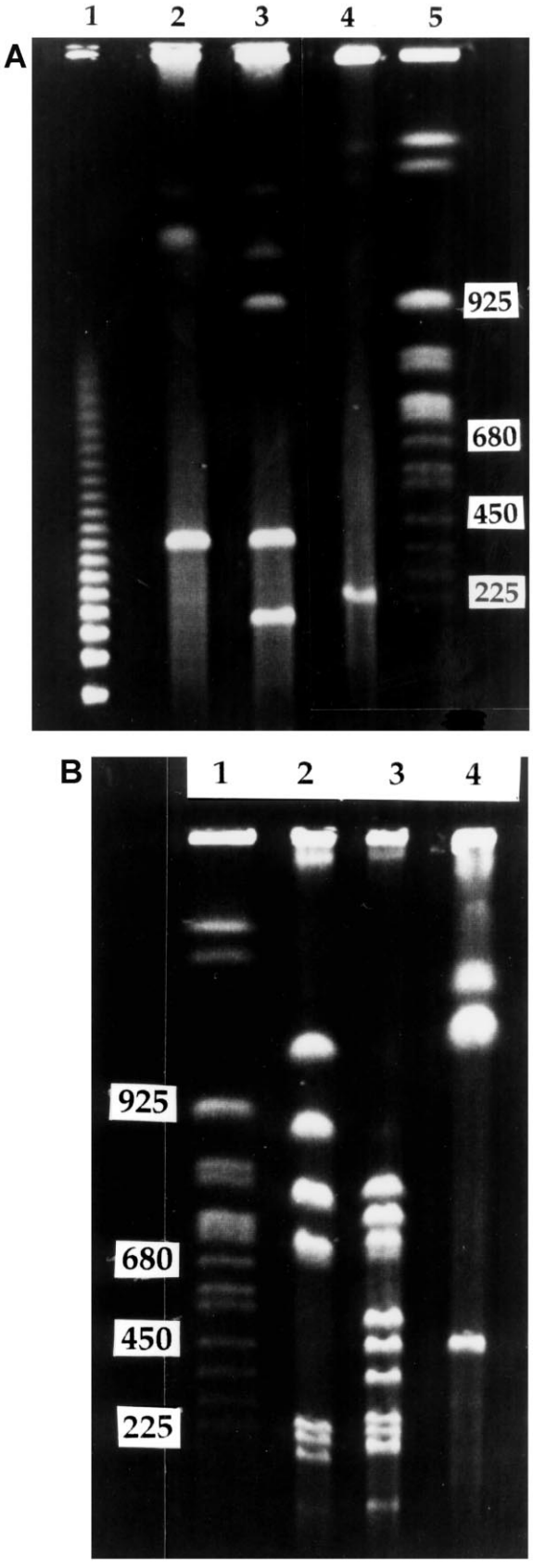
The MS-1 *magA* gene sequence was isolated by PCR using oligos 5′-GAAGACGGCCATCTTCACCA-3′ and 5′-TTCTTCCAGATGAACTTGAA-3′, followed by inverse PCR of a *Sac*II fragment using oligos 5′-CAGGTTCCAGCCCAAACCGT-3′ and 5′-ATCGGCAGCGTGCTGTTGAT-3′. Partial sequences of the AMB-1 *bfr* genes were obtained using oligos 5′-CGCATGTATGAAGACTGGGG-3′ and 5′-TGCGTTTCCAGCCAGTCGAT-3′, followed by inverse PCR of an *Msp*I digest with oligos 5′ GAGATCATCGCCAACGATCT-3′ and 5′-TCATCTCCTCGATGGACAGC-3′. Partial sequences of the Ai *bfr* genes were obtained using the same first two oligos as for AMB-1 and then completing the sequence with oligos 5′-TTCAAGAAACAGCA-CCCGCT-3′ and 5′-TGTGGAATACGAGGTGACTG-3′. The GenBank Accession Numbers are AF257521, AF271380, and AF268910, respectively.

Cloned genes were sequenced by the DNA Sequencing Core Facility at Caltech, using M13F and R oligos, and identified by homology searches using the BLAST network service of the National Center for Biotechnology Information. The maximum *E* value considered to be significant was 10^{-5}.

3. Results

3.1. Genome size

Genome size was determined by cleaving MS-1 genomic DNA into relatively few fragments with restriction enzymes and separating the fragments by PFGE. Since the DNA of MS-1 is 64% GC (Blakemore et al., 1979), it is necessary to use restriction enzymes such as *Pac*I, *Pme*I, and *Swa*I,



which recognize 8 bp-AT-rich targets. Both single and double digests were made and gels were run under different conditions and with different switch times as required. Pertinent examples are given in what follows.

Although MS-1 DNA is usually poorly digested by *Pac*I, the enzyme regularly produces one band (Pc2) of 230 kb (Fig. 1A, lane 4). As will be discussed, this band has two features important to the subsequent analysis.

Digestion of the DNA with the enzyme *Pme*I appeared at first to yield three bands (Pm) of 375, 1250 and 1500 kb (Fig. 1A, lane 2 and Fig. 1B, lane 4). The 1250 band, however, was always more intensely stained than the other bands of similar size, suggesting that it might consist of two overlapping bands of about the same size. This was confirmed in double digests with *Pac*I, which resolved the 1250 kb region into two smaller bands (Fig. 1A, lane 3). The result of the digestion with *Pme*I, therefore, is actually four bands of 375, 1250, 1250, and 1500 kb. The double digest produced a total of six fragments (PmPc), including two that are smaller than the *Pac*I fragment (Fig. 1A, lane 3, see bottom of gel for smallest band), suggesting that this fragment is in turn cut by *Pme*I. This would be consistent if the *Pac*I sites lay on either side of the junction between Pm2 and Pm3. Thus, this result provides the information that fragments Pm2 and Pm3 are contiguous.

Digestion with the enzyme *Swa*I produces seven bands (Sw), ranging in size from 130 to 1200 kb. Double digests using *SwaI* and *PmeI* (Fig. 1B, lane 3) produced twelve fragments (SwPm) ranging from 40 to 900 kb.

The fragments from the different digests in Table 1 add up to 4255, 4375, 4360, and 4400 kb, respectively, giving an average of 4348 kb for the genome size.

3.2. Physical structure

In general, PFGE is used to separate linear molecules of DNA. Large circular molecules are unable to enter the gel (Birren and Lai, 1993). This was used as a test for circularity of the MS-1 genome. The mobility of undigested MS-1 genomic DNA, prepared for PFGE, was compared with the 4.8 mb circular DNA of Ec, or the three linear chromosomes of Sp, which are 3.5, 4.6, and 5.7 mb. Under the conditions where all of the Sp chromosomes moved into the gel, neither the MS-1 nor Ec DNA did so (Fig. 2)

Fig. 1. Pulsed-field gel analysis of MS-1 genomic DNA digested with restriction enzymes. One percent agarose gels, loaded with single plugs of MS-1 DNA prepared and digested as described in Sections 2.2 and 2.3, were run in 0.5 × TBE buffer at 6 v/cm^2 for 24 h with a switch time of 60 to 120 s. Part A, digestion with *Pac*I and *Pme*I: Lane 1, lambda ladder; lane 2, digestion with *Pme*I only; lane 3, digestion with *Pme*I and *Pac*I; lane 4, digestion with *Pac*I only; lane 5, Sc chromosomes with the molecular weights (kb) of some of the chromosomes indicated. Part B, digestion with *Pme*I and *Swa*I: Lane 1, Sc chromosomes as in part A; lane 2, digestion with *Swa*I only; lane 3, digestion with *Pme*I and *Swa*I; lane 4, digestion with *Pme*I only.

Table 1
Sizes of bands produced by digestion of MS-1 genomic DNA[a]

PacI		PmeI		SwaI		PmeI + PacI		SwaI + PmeI	
Band	kb	Band	kb	Band	kb	Band	kb	Band	kb
1	?	1	1500	1	1200	1	1500	1	885
2	230	2	1250	2	1000	2	1200	2	800
		3	1250	3	835	3	1000	3	710
		4	425	4	710	4	425	4	500
			4425	5	210	5	210	5	425
				6	170	6	40	6	325
				7	130		4375	7	210
					4255			8	170
								9	140
								10	130
								11	40
									4335

[a] Plugs of MS-1 genomic DNA were digested with restriction enzymes *Pac*I, *Pme*I, or *Swa*I, either singly or doubly, as indicated, and the fragments separated by PFGE, as described in Sections 2.2–2.4. The fragments produced and the sizes (kb) of the bands, estimated by comparison with the 50 kb lambda ladder and Sc chromosome molecular weight markers, are listed. In what follows, the fragments are numbered from the largest to the smallest and referred to as Pc, Pm, Sw, PmPc, or SwPm for the single or double digests.

suggesting that the MS-1 chromosome is circular in structure, like that of Ec.

Although no plasmid-like structures were seen on gels of undigested DNA or in conventional plasmid DNA preparations, faint bands less than 100 kb in size were occasionally seen following digestion of the DNA with restriction enzymes. One of these (Fig. 3, lane 3), produced by digestion with *Swa*I, is discussed in more detail in later sections.

3.3. Construction of a physical map

Following electrophoresis of the digests, the pulsed-field gels were blotted onto nylon membranes and probed with ^{32}P-labeled cloned MS-1 DNA (example in Fig. 3). The probes included some previously-identified genes, as well as a number of uncharacterized clones from *Eco*RI (R) and *Hind*III (H) libraries. The localization of each probe to the bands produced by single digestion with *Pac*I, *Pme*I or *Swa*I or by double digestion with *Pme*I and *Swa*I is given in Table 2.

Two of the probes reacted with the barely visible 40 kb-band that only appears following *Swa*I digestion (Fig. 3, lanes 4 and 5). The possibility that this band represents an extra-chromosomal structure is taken up in the discussion. It has not been included in the map.

Using the probes as guides, the fragments produced by *Pme*I, can be connected in the following way (see Fig. 4): the largest *Swa*I band overlaps Pm1 and Pm3 and includes Pm4 so that the *Pme*I bands are most likely aligned: Pm1-Pm4-Pm3. As mentioned earlier (Section 3.1), the small *Pac*I fragment overlaps bands Pm2 and Pm3, resulting in the alignment Pm1-Pm4-Pm3-Pm2. As yet, there is no evidence for the physical linking of Pm1 and Pm2, as would be expected if the genome were circular. The linking may be through the 140 kb (SwPm9) band produced by a *Swa*I/*Pme*I double digest, which is the only fragment that still lacks an identifying probe. A linear 4.3 mb genome, however, would surely have been seen in the analysis of undigested DNA discussed in Section 3.2. Two of the smaller bands produced by *Swa*I, 5 and 7, have not been unambiguously positioned as yet.

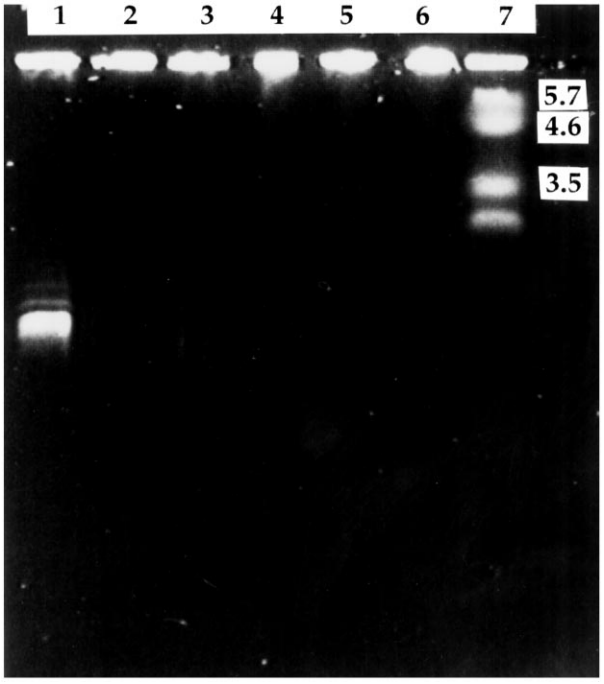


Fig. 2. Pulsed-field gels analysis of undigested MS-1 genomic DNA. One percent agarose gels were loaded with plugs of undigested, genomic MS-1 DNA and run in 1 × TAE buffer at 2 v/cm^2 for 72 h with a switch time of 2100 s. Lane 1, Sc chromosomes; lane 2, Ec DNA; lanes 3–5, MS-1 DNA; lane 6, Ad DNA; lane 7, Sp chromosomes, with the molecular weights (mb) indicated.

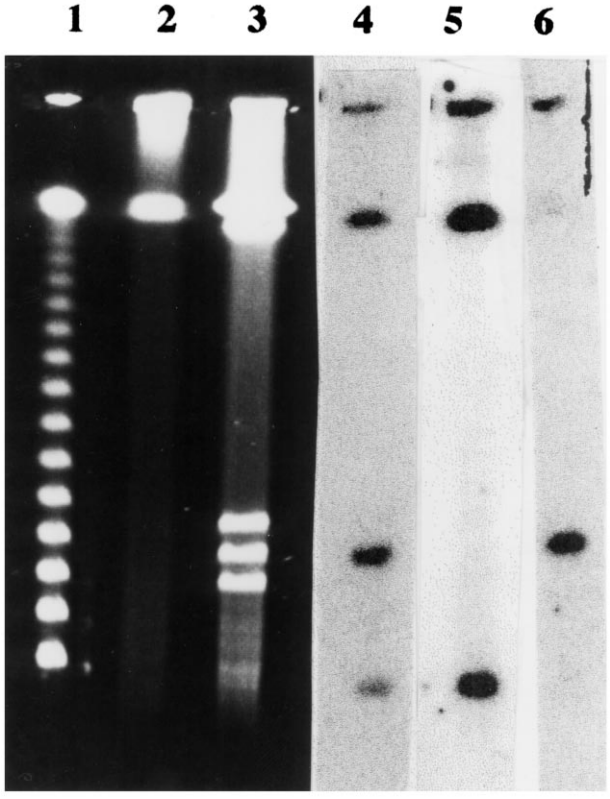


Fig. 3. Localization of genes to an extra-chromosomal structure. Plugs of MS-1 DNA were digested with the restriction enzyme *Swa*I and subjected to PFGE, using a run time of 20 h and a switch time of 13–54 s. The gel, minus the lambda ladder, was subsequently blotted onto a nylon membrane (see Section 2.5), which was probed sequentially with α^{32}P-dCTP-labelled insert DNA. The radioactivity was stripped off between probes. Lane 1, lambda 50 kb ladder; lane 2, undigested DNA; lane 3, *Swa*I-digested DNA; lanes 4–6, *Swa*I-digested DNA shown in lane 3, probed with partial sequences of the 16S rRNA gene, the *bra* and *por* genes, and *bfr* genes 1 and 2, respectively.

With these limitations in mind, preliminary restriction maps for the *Pme*I and *Swa*I enzymes have been prepared (Fig. 4). The *dna*A gene was localized to the small *Pac*I fragment, Pc2 (Table 2), which earlier (Section 3.1) had been found to connect *Pme*I bands 2 and 3. Since the *dnaA* gene is known to be involved in DNA replication and is usually located near the origin of replication (McMacken et al., 1987), the junction between *Pme*I fragments 2 and 3 is taken to define the origin of replication of the chromosome and the map is displayed with the Pm2/Pm3 junction at the zero position.

3.4. Organization of the bfr and magA genes

Strain MS-1 was found (Bertani et al., 1997) to have two *bfr* genes in overlapping configuration. Since there are, as yet, relatively few examples of organisms that encode two Bfr subunits, we have extended these results to two other organisms, the closely related (Burgess et al., 1993), magnetotactic strain AMB-1 and the non-magnetic relative, Ai. As

Table 2
Localization of specific genes or clones to restriction enzyme fragments of the MS-1 genome[a]

Gene[b] or clone	Location
H2A2, H6A6	Sw7, Pm2, SwPm10
nifL1, pntA, rpl6, rpoA, R8B4	Sw2, Pm2, SwPm1
ftsH, nifL2, H16A3	Sw5, Pm1, SwPm7
bra, napA, por, rrn2, secF, sodB, R8B1	Sw4, Pm1, SwPm3
dms, fdx, H16A8	Sw1, Pm1, SwPm6
stp3, stp4, H6A4	Sw1, Pm4, SwPm5
stp5, magA, rnhII, trpB	Sw1, Pm3, SwPm4
bfr1, bfr2, stp1, rrn1	Sw6, Pm3, SwPm8
gtp, stp2	Sw3, Pm3, SwPm2
dnaA, ech, lrp	Sw3, Pm3, SwPm2, Pc2

[a] Pulsed-field gels of digested MS-1 DNA were blotted onto nylon membranes and probed with cloned MS-1 DNA, described in Section 2.5.
[b] Inserts were partially sequenced and identified as described in Section 2.6.

can be seen in Fig. 5, the former has the same one nucleotide-overlap seen in the *bfr* genes of MS-1, whereas the latter has two *bfr* genes in the same order, but with 16 bp separating the ORFs.

MS-1 and AMB-1 also share similarities in the organization of the *magA* gene. The *magA* gene of strain AMB-1 overlaps a downstream gene, encoding RNaseHII, by 24 nucleotides (Matsunaga et al., 1997) and the *magA* gene of MS-1 (see AF257521) shows the same arrangement.

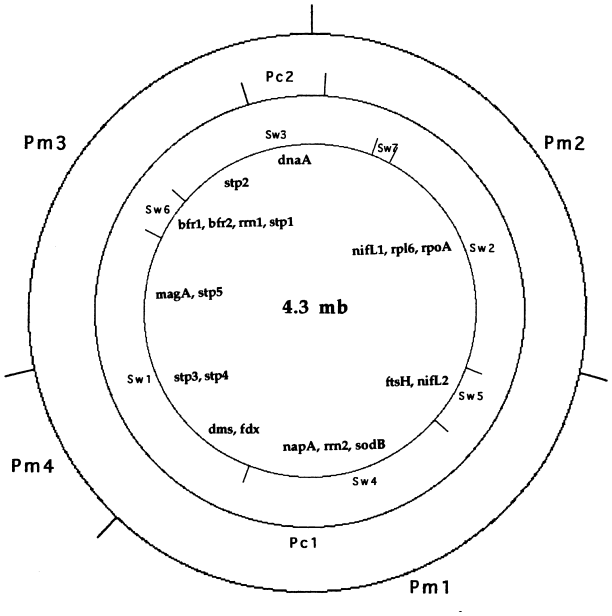


Fig. 4. Restriction and genetic maps of the MS-1 genome. Using the data in Tables 1 and 2, a restriction map produced by digestion of MS-1 DNA with the restriction enzyme *Pme*I is presented as 4.3 mb circle. The most probable arrangements of restriction fragments produced with enzymes *Pac*I and *Swa*I are displayed as concentric circles within the *Pme*I map. The locations of some of the genes listed in Table 2 are indicated.

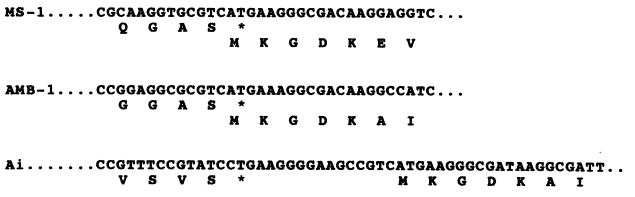


Fig. 5. Organization of the *bfr* genes in strains MS-1 and AMB-1 compared to Ai. Partial nucleotide sequences showing the positions of the C-terminal ends of the genes encoding the Bfr2 subunits relative to the genes encoding the N-terminal ends of the Bfr1 subunits in the strains MS-1 and AMB-1 and Ai are displayed. The sequences of the putative proteins are given below the nucleotide sequences. Additional sequence is available from GenBank using accession nrs. AF001959, AF271380, and AF268910, respectively.

4. Discussion

PFGE analysis of the MS-1 genome suggests that it is a circular structure of about 4.3 mb. Although no extra-chromosomal structures are visible in undigested DNA, the radioactive probes for 16S rRNA and the *bra* and *por* genes both react with a barely visible 40 kb-band that only appears following *Swa*I digestion (Fig. 3, lane 4). The nature of the minor band is not clear. Since the 40 kb-band appears only following digestion with *Swa*I, it could represent a circular plasmid containing these genes that is linearized by *Swa*I digestion. Another possibility is that it is derived by secondary activity of the *Swa*I enzyme from band Sw4, which also contains the 16S rRNA, *bra*, and *por*genes. This is the only case, so far, where we have found any genetic features associated with a possibly extra-chromosomal structure.

MS-1 has been described as an organism that carries out dissimilatory nitrate reduction under microaerophilic conditions (Escalante-Semerena et al., 1980). In addition, Blakemore et al. suggested (1979), that it might be capable of fixing atmospheric nitrogen. In agreement with these observations, the genes of MS-1 that were identified by partial sequencing of the probes (Table 2) used to construct the physical map, include *napA*, a gene encoding a subunit of the protein involved in dissimilatory nitrate reduction, and two *nifL* analogs, encoding components of a nitrogen-fixing system. Typical eubacterial analogs of genes involved in DNA (*dnaA*), RNA (*rpoA*) and protein synthesis (*rpl6*, *rrn*), as well as cell division (*ftsH*), have also been found. Interestingly, five (*stp*) out of the twenty-seven genes were identified as the sensory part of a two-component sensory transduction system.

One of our motives for undertaking this study was to determine if the genes involved in magnetite synthesis - *bfr* and *mag*A - were clustered in strain MS-1. As can be seen from Table 2 and Fig. 4, the *bfr* genes map to the 170 kb band, Sw6, and *mag*A maps to the adjacent 500 kb overlap region, SwPm4. Thus, these two genes occupy the same 670 kb segment, or 17%, of the genome at a maximum and are probably closer than that. In addition, the genes encoding various sensory transduction systems, mentioned above, are located in the same third of the genome. Although not the tight clustering that we had hoped to find, these genes delineate a specific region that should be worth investigating further.

MS-1 Bfr has two unusual features. Unlike the case for model organism Ec and most of the other entries in GenBank, it is composed of two subunits, rather than one. In addition, in the few GenBank organisms that do produce two Bfr subunits, the genes encoding the two subunits are not overlapping, whereas in MS-1, they overlap by one nucleotide. The function of the overlap might be to keep the two genes together or it might play a regulatory role in determining the relative amounts of the subunits that are made. The subunits are produced as individual proteins from cloned MS-1 DNA expressed in an Ec host (unpublished results).

We have extended these observations to two additional organisms, the magnetic strain, AMB-1, and a non-magnetic relative, Ai. The two *Magnetospirillum* strains are very closely related - Burgess et al. (1993) found only 8 bp out of 1424 of the 16S rDNA of MS-1 and AMB-1 to be different – whereas there are 96 differences out of 878 bp between MS-1 and Ai (unpublished results). As shown in Fig. 5, the arrangement of the *bfr* genes in MS-1 and AMB-1 is identical. In Ai, however, although the genes for the two subunits are in the same order (*bfr*2 upstream of *bfr*1), they are separated by 16 bp. The relevance of the overlapping *bfr* genes to magnetite formation is under study.

5. Note added in proof

In the meantime, a draft sequence of the MS-1 genome has been carried out by the Joint Genome Institute and is available at their web site: http://www.jgi.doe.gov/ under the JGI Microbial Genomics link.

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