Frequent Mutations within the Genomic Magnetosome Island of *Magnetospirillum gryphiswaldense* Are Mediated by RecA^{▽†}

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Genes for magnetosome formation in magnetotactic bacteria are clustered in large genomic magnetosome islands (MAI). Spontaneous deletions and rearrangements were frequently observed within these regions upon metabolic stress. This instability was speculated to be due to RecA-dependent homologous recombination between the numerous sequence repeats present within the MAI. Here we show that a RecA-deficient strain of *Magnetospirillum gryphiswaldense* (IK-1) no longer exhibits genetic instability of magnetosome formation. Strain IK-1 displayed higher sensitivity to oxygen and UV irradiation. Furthermore, the lack of RecA abolished allelic exchange in the mutant. Cells of strain IK-1 displayed a slightly altered (i.e., more elongated) morphology, whereas the absence of RecA did not affect the ability to synthesize wild-type-like magnetosomes. Our data provide evidence that the observed genetic instability of magnetosome formation in the wild type is due predominantly to RecA-mediated recombination. In addition, increased genetic stability could make strain IK-1 a useful tool for the expression of genes and further genetic engineering, as well as for biotechnological production of bacterial magnetosomes.

The magnetotactic alphaproteobacterium *Magnetospirillum gryphiswaldense* synthesizes magnetosomes, which consist of magnetite (Fe₃O₄) crystals enclosed within intracytoplasmic vesicles of the magnetosome membrane (MM) (35). All of the genes known to be responsible for magnetosome formation were found clustered within a conspicuous genomic “magnetosome island” (MAI) which comprises the *mamAB*, *mamGFDC*, *mms6*, and *mamXY* operons (33, 40, 41). Genes of these operons are involved in MM vesicle formation, magnetite biomineralization, and chain assembly (22, 30, 32, 34). The presence of conserved MAI-like structures in other cultivated (15, 16, 24, 27, 40) and uncultivated magnetotactic bacteria (MTB) (17, 18) suggests that the MAI was horizontally transferred between distantly related bacteria (15). In addition to all of the identified magnetosome genes, the MAI harbors numerous transposase genes, as well as hypothetical genes with unknown functions. It was shown in previous studies that the MAI undergoes frequent rearrangements during subcultivation in the laboratory (32, 40). Spontaneous mutants affected in magnetosome formation accumulated with a frequency of up to 10^{−2} after prolonged storage or exposure to oxidative stress and had all lost partial or complete *mms* and *mam* gene clusters encoding magnetosome proteins, as well as various copies of sequence repeats (40). This led to the hypothesis that the observed instability was caused by RecA-mediated homol-

ogous recombination, as also observed in other organisms like *Mycobacterium bovis* (29).

RecA is a ubiquitous and highly conserved protein. In *Escherichia coli*, RecA catalyzes strand exchanges between homologous DNA molecules via RecA–single-stranded DNA (ssDNA) complexes. Furthermore, it also plays a key role in signal transduction following DNA damage. Therefore, RecA binds ssDNA generated by DNA damage. The activated RecA complex then induces the SOS repair functions (25). Loss of RecA is associated with higher sensitivity to DNA damage and severe growth and recombination deficiency phenotypes (6–8, 23, 39). In two early studies, the *recA* gene of *M. magnetotacticum* MS-1 was shown to complement a *recA*-deficient *E. coli* strain with respect to recombination and DNA repair deficiency (2, 3). However, nothing is known about the physiological function of RecA in MTB and its involvement in the observed genetic rearrangements.

In this study, we constructed a RecA-deficient mutant strain of *M. gryphiswaldense*. Compared to the wild type (WT), strain IK-1 displays increased UV and oxygen sensitivity and decreased homologous recombination ability. Furthermore, the mutant no longer exhibits spontaneous mutations within MAI genes, indicating that the genetic instability of the WT depends on RecA activity. Its increased genetic stability may render the *recA* mutant strain a useful tool for gene expression and genetic engineering of *M. gryphiswaldense*.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The bacterial strains and plasmids used in this study are shown in Table 1. *E. coli* strains were cultivated in lysogeny broth (4) supplemented with 25 µg/ml kanamycin (Km), 12 µg/ml tetracycline (Tet), and 15 µg/ml gentamicin as previously described (16). For growth of *E. coli* BW29427 (kindly provided by B. Wanner, Purdue University, West Lafayette, IN), LB was supplemented with DL-α,ε-diaminopimelic acid (Sigma-Aldrich, Steinheim, Germany) to a final concentration of 1 mM. Liquid cultures and single colonies of *Magnetospirillum* strains were cultivated in FSM

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Characteristic(s)	Reference(s) or source
Strains		
<i>M. gryphiswaldense</i>		
MSR-1 R3/S1	WT (spontaneous Rif ^r Sm ^r mutant)	37
MSR-1 IK-1	R3/S1 $\Delta recA$	This study
<i>E. coli</i>		
DH5 α	F ⁻ ϕ 80lacZ Δ M15 Δ (lacZYA-argF)U169 <i>recA1 endA1 hsdR17</i> (r _K ⁻ m _K ⁺) <i>phoA</i> <i>supE44</i> λ^- <i>thi-1</i> <i>gyrA96</i> <i>relA1</i>	Invitrogen
BW29427	<i>dap</i> mutant auxotrophic derivative of <i>E. coli</i> strain B2155	K. Datsenko and B. L. Wanner, unpublished data
Plasmids		
pJet 1.2/blunt	Ap ^r , <i>eco47IR</i> (lethal restriction enzyme gene), <i>rep</i> (pMB-1)	Fermentas
pJetIB013+014	pJet containing the upstream fragment of the <i>recA</i> gene	This study
pJetIB015+016	pJet containing the downstream fragment of the <i>recA</i> gene	This study
pCM184	Ap ^r Km ^r Tet ^r ; broad-host-range allelic exchange vector	20
pCM184+ <i>recA</i> flank	pCM184 with 1.6- and 1.8-kb up- and downstream fragments of <i>recA</i>	This study
pCM157	Tet ^r ; Cre recombinase expression vector	20
pBBR1MCS5	Km ^r ; mobilizable broad-host-range vector	19
pBBR1MCS5 <i>recA</i>	pBBR1MCS5 containing <i>recA</i>	This study
pAL01	pK19mobGII vector (Km ^r , pMB-1 replicon, <i>gusA</i> , <i>lacZ</i>) containing a 2-kb fragment upstream of <i>mgr4019</i>	A. Lohße, unpublished data

medium (13) or on agar plates incubated at 30°C under aerobic, microaerobic, or anaerobic conditions. For aerobic growth, cells were cultivated in free gas exchange with air. For microaerobic and anaerobic conditions, flasks were sealed with butyl rubber stoppers and flushed before autoclaving with an atmosphere of 1% O₂-99% N₂ (microaerobic conditions) or N₂ (anaerobic conditions). Liquid cultures were agitated at 130 rpm under aerobic and microaerobic conditions. Agar plates were incubated in anaerobic jars in a 1% O₂-99% N₂ or N₂ atmosphere. For growth from single colonies, cells were transferred into 100 μ l FSM medium in 96-deep-well plates (Eppendorf, Hamburg, Germany). Plates were incubated in anaerobic jars for 5 to 6 days. The volume was gradually increased by the addition of 200 μ l to a final volume of 800 μ l.

Molecular, biological, and genetic techniques. Unless specified otherwise, standard protocols were used as described previously (28). DNA was sequenced using BigDye Terminator v3.1 chemistry on an in-house ABI 3700 capillary sequencer. Sequences were analyzed with the Vector NTI (Invitrogen) program. Primers (see Table S1 in the supplemental material) were purchased from Sigma-Aldrich (Steinheim, Germany). Conjugation experiments were performed as described before (37, 38), with the following modifications. MSR-1 cells (2×10^9) were mixed with strain BW29427 cells and incubated microaerobically for 12 h on activated charcoal agar medium. Cells were rinsed from the agar surface, and a 1:5 dilution was plated on FSM agar supplemented with appropriate antibiotics. Transconjugants were screened after incubation for 7 to 10 days in anaerobic jars under microaerobic conditions.

Analytical methods. The optical density at 565 nm (OD₅₆₅) and magnetic response (C_{mag}) of *M. gryphiswaldense* cultures were measured turbidimetrically as previously described (36, 47). Intracellular iron concentrations were measured after incubation under anaerobic conditions using a modified version of the ferrozine assay (42). One-milliliter cultures were centrifuged for 1 min at 11,000 rpm and resuspended in 90 μ l HNO₃ (65%) for 3 h at 99°C. Afterwards, the lysate was cleared by centrifugation and resuspended in 50 μ l ammonium acetate.

Transmission electron microscopy (TEM) was performed as previously described (16). For differential interference contrast (DIC) microscopy using an Olympus IX81 inverted microscope, 5 μ l of liquid culture was fixed on an agar pad.

Generation of *recA* deletion strain IK-1. A *cre-lox*-based method was used (20, 30, 41) to generate an unmarked $\Delta recA$ mutant strain as described elsewhere (see Fig. S1 in the supplemental material). First, 1.8- and 1.6-kb fragments up- and downstream of the *recA* gene (*mgr2512*) were amplified, respectively, with Phusion polymerase (NEB GmbH, Frankfurt am Main, Germany) and cloned into the pJet 1.2/blunt vector (Fermentas GmbH, St. Leon-Rot, Germany) to yield pJetIB013+014 (pJet containing the upstream fragment) and pJetIB015+016 (pJet carrying the downstream fragment). Plasmid pJetIB013+014 was digested

with BglII and NcoI, and the 1.8-kb upstream fragment was inserted into suicide plasmid pCM184. The resulting plasmid, pCM184+IB013+014, and pJetIB015+016 similarly digested with MluI and AgeI were religated to yield pCM184*recA*flank. After verification by PCR (primers IB013 and IB014 and primers IB016 and IB017), the plasmid was transferred into *M. gryphiswaldense* R3/S1 (WT) by conjugation. Correct insertion was verified via PCR using primers IB017 and IB018, IB013 and 54rv, or IB014 and 61fw, respectively. In the last step, putative deletions were checked via Southern blot analysis (see Fig. S1E in the supplemental material). For Southern blot analysis, 5 μ g of KpnI-digested genomic WT or $\Delta recA$ DNA was hybridized with a [α -³²P]dATP-labeled *recA* probe (primers IB017 and IB018) and excision of the Km resistance cassette was performed by transferring plasmid pCM157 expressing Cre recombinases. One positive clone was cured of plasmid pCM157 by passaging the cells 10 times in FSM medium in the absence of Km selection. Loss of the Km resistance cassette was verified via PCR and spotting on FSM-Km agar. The mutant strain was named *M. gryphiswaldense* IK-1.

UV irradiation assay. At an OD₅₆₅ of 0.15, 20-ml volumes of *M. gryphiswaldense* cultures were harvested and washed twice in 10 ml MgSO₄ (0.1 M). A 5-ml volume of the suspension were transferred into sterile petri dishes (lids removed). After empirical testing of different irradiation intensities in a Biolink DNA cross-linker (Biometra GmbH, Göttingen, Germany), a dose of 15 mJ/cm² was considered to be most appropriate. After UV irradiation, cells were harvested, resuspended in 10 ml FSM medium, and incubated for 12 h at 30°C. The OD₅₆₅ was adjusted to 0.04, and 1-ml aliquots were plated on FSM agar. Colonies were counted after 7 days of microaerobic incubation.

Induction and screening of MAI mutants. Twelve clones each of the WT and strain IK-1 were incubated in 100 μ l FSM within the microwells of a 96-well plate (Sigma-Aldrich, Steinheim, Germany) at 4°C under microaerobic conditions for 1 week (see Fig. S2 in the supplemental material). This was followed by six cycles of growth to saturation for 48 h per cycle (without shaking) at 30°C under microaerobic conditions. Subsequently, 5 μ l of each of the 12 cultures was spotted onto FSM agar (250 μ M Fe) in several dilutions (1/10 to 1/100,000), incubated at 30°C under anaerobic conditions, and then visually screened for the appearance of white colonies. Twenty-four white colonies were randomly picked and cultured for further investigations.

Recombination assay. The suicide plasmid pAL01, carrying a region homologous to a 2-kb sequence within the MAI (upstream of *mgr4019*), was transferred into the WT, strain IK-1, and transcomplemented strain IK-1+/pBBR1MCS5*recA* via conjugation. We used 4×10^8 recipient cells per conjugation experiment. Colonies were counted after 7 days of incubation, and recombination efficiency was calculated.

RESULTS

Construction and characterization of *ΔrecA* mutant *M. gryphiswaldense* strain IK-1. The *recA* gene from *M. gryphiswaldense* (NCBI accession no. CU459003) is located on contig1066 of the partial genome sequence (27). The predicted protein sequence (358 amino acids; molecular mass, 38 kDa) is 91% identical to that of *M. magneticum* AMB-1 and 68% identical to that of *E. coli*. Downstream, *recA* is adjacent to a gene encoding a putative sensor histidine kinase (*mgr2511*), and upstream, it is adjacent to *secA* (*mgr2513*), encoding a putative translocator protein (see Fig. S1A in the supplemental material). Similar to the organization in other bacteria like *E. coli* (14), *recA* of *M. gryphiswaldense* is likely to be monocistronic.

To analyze the function of RecA, deletion mutant strain *M. gryphiswaldense* IK-1 was constructed by allelic exchange. IK-1 showed growth characteristics similar to those of the WT under microaerobic and anaerobic conditions (Fig. 1), whereas its doubling time was increased under aerobic incubation conditions (WT doubling time, 5.0 h; IK-1 doubling time, 7.4 h). The C_{mag} values of IK-1 were lower than those of the WT under microaerobic and anaerobic conditions (WT microaerobic C_{mag} , 1.4; IK-1 microaerobic C_{mag} , 0.9; WT anaerobic C_{mag} , 1.5, IK-1 anaerobic C_{mag} , 0.9). However, strain IK-1 had an intracellular iron content similar to that of the WT (WT, 3.5% of dry weight; IK-1, 3.9% of dry weight) and similar sizes (Mann-Whitney P value, ≥ 0.05) and numbers (WT, 29; IK-1, 27) of crystals per cell (Fig. 2A and D). TEM (Fig. 2D) and DIC (Fig. 2B and C) analyses revealed a variable proportion of elongated and small vibrioid cells. Fifty-eight percent of the cells of the mutant were smaller than 3 μ m, 38% of the larger cells (3 to 10 μ m) were spiral shaped, and 2% of the cells were aberrantly elongated (≥ 10 μ m) under microaerobic conditions after 24 h of incubation. In contrast, WT cultures contained 41% short vibrioid cells, 58% larger spiral-shaped cells, and 0.16% aberrantly long cells. Under aerobic conditions, the numbers of elongated cells of both IK-1 (13%) and WT (2%) were increased, consistent with a decreased number of short vibrioid cells (IK-1, 6.4%; WT, 2%). Also, if the bacteria were incubated under anaerobic conditions at 25°C, i.e., in the absence of oxidative and temperature stress, the percentage of elongated cells was still higher in the mutant (4% and 1%, respectively).

IK-1 is more sensitive to UV light. Since *recA* deletion mutants of other bacteria were shown to be more sensitive to UV light exposure (1, 26, 44) due to their inability to induce an SOS response (21, 43), we performed irradiation assays. UV sensitivity, measured by determining the number of surviving CFU/ml, was 28-fold higher in the mutant than in the WT (Fig. 3B). Whereas the WT count was 1.38×10^3 CFU/ml (survival rate, 7.8×10^{-5}) after irradiation, that of the mutant under the same conditions was 49.7 CFU/ml (survival rate, 4.1×10^{-6}). Increased UV sensitivity could be partially restored by providing the WT *recA* gene in *trans* on plasmid pBBR1MCS5*recA* (survival rate, 1.78×10^{-5} ; 2.13×10^2 CFU/ml).

IK-1 is impaired in homologous recombination. To test whether homologous recombination was impaired in IK-1, the chromosomal integration of a suicide plasmid (pAL01) carrying a region homologous to a 2-kb chromosomal fragment was

FIG. 1. Growth (OD_{565}) and C_{mag} of *M. gryphiswaldense* IK-1 and the WT (●, WT C_{mag} ; ○, IK-1 C_{mag} ; ▲, WT OD_{565} ; △, IK-1 OD_{565}) during incubation for 30 h under microaerobic (A), aerobic (B), and anaerobic (C) conditions.

investigated (Fig. 3A). Kan^r transconjugants were counted, and recombination efficiency was calculated by considering the number of recipient cells per conjugation. Whereas only very few Km^r colonies (a total of four clones) were obtained in the *ΔrecA* background (efficiency, 3.3×10^{-9}), the efficiency of the WT strain was 80-fold higher (2.7×10^{-7}). Recombination efficiency was restored to the WT level in the transcomplemented mutant strain IK-1+/pBBR1MCS5*recA* (3×10^{-7}).

FIG. 2. (A) Magnetosome size distributions under anaerobic conditions (Mann-Whitney P value, ≥ 0.05) in IK-1 and the WT. (B) Distributions of different cell sizes of strain IK-1 and the WT were estimated. Morphotypes were investigated under aerobic, microaerobic, and anaerobic conditions. (C) DIC micrographs showing representative short, medium, and long cells. (D) TEM of the WT and strain IK-1. For TEM pictures, cells were incubated at 30°C under microaerobic conditions.

***M. gryphiswaldense* IK-1 displays increased genetic stability of the MAI under physiological stress conditions.** To assess the occurrence of spontaneous mutants in the WT and strain IK-1, we established an incubation regime for the efficient induction and isolation of mutants with either a weakly magnetic or a nonmagnetic phenotype (see Fig. S2 in the supplemental material). Cells were stored at 4°C for 1 week under microaerobic conditions. Afterwards, aliquots were spotted onto plates and colonies of the mutant strain and the WT were enumerated. Under oxygen-limited or anaerobic conditions, magnetite-synthesizing colonies of *M. gryphiswaldense* are dark brown (Mag^+), whereas clones which have lost the ability to produce magnetite (Mag^-) can be clearly distinguished by their whiteness (32) (see Fig. S2B). While no white clones were detectable

among >10,000 clones of strain IK-1, 22% (60 out of 267) clones of the WT were white and thus seemed to be weakly magnetic or nonmagnetic. Of 24 randomly picked white clones, 18 proved to be completely nonmagnetic (Mag^-) upon subcultivation, whereas the C_{mag} of 5 clones was decreased (0.01 to 0.4) and 1 clone had a WT-like C_{mag} of 1.7 (Mag^+). All 18 Mag^- mutants were screened by PCR for deletions or rearrangements within the *mamAB* operon, which was previously shown to be essential for magnetosome formation in *Magnetospirillum* (22, 41). We failed to amplify fragments of the expected size from 11 Mag^- clones for at least one of the following gene regions: *mamH-mamE*, *mamM-mamN*, *mamO-mamA*, *mamQ-mamS*, *mamT-mamU*, and *mamJ-mamL*.

In six clones, the *mamH-mamE* region was absent, whereas

FIG. 3. (A) Recombination assay results. A suicide vector carrying a 2-kb insert homologous to a partial region of the MAI was transferred into the WT and the *recA* mutant. The frequency of cells with genomic insertions was estimated by the number of Km-resistant clones after 7 days of microaerobic incubation (4×10^8 recipient cells per conjugation). (B) UV irradiation assay results. Cells were irradiated with 15 mJ/cm² UV light. Both assays were performed in triplicates. Survival rates (CFU/ml) after irradiation were estimated after 7 days of microaerobic incubation.

in clones 8 and 15, a product 1 kb larger than expected was amplified. We failed to amplify a product of the expected size for *mamJ-mamL* from five clones, whereas in two of them (clones 10 and 12), the size of the PCR product was about 2 kb larger than that obtained from the WT, which is consistent with previous observations that *mamJ*, which harbors a repetitive and hypervariable domain, undergoes frequent rearrangements (32). The *mamO-mamA* region was absent from six clones, and *mamQ-mamS* was absent from five clones (Table 2; see Fig. S3 in the supplemental material). The *mamT-mamU*

region was not detectable in two clones, whereas *mamM-mamN* seemed to be present in all of the spontaneous mutants. No modifications within the *mamAB* operon of seven Mag⁻ mutants could be detected via PCR.

DISCUSSION

Analysis of RecA in MTB has so far been limited to the complementation of an *E. coli recA* mutant by the *M. magnetotacticum* gene (2). In this study, we generated and analyzed

TABLE 2. Results of PCR amplification of various regions within the *mamAB* operon from the spontaneous Mag⁻ mutants of the WT strain^a

Clone	Presence of following amplification product:					
	<i>mamH-mamE</i> (3.8 kb)	<i>mamJ-mamL</i> (2.7 kb)	<i>mamM-mamN</i> (2.2 kb)	<i>mamO-mamA</i> (3.4 kb)	<i>mamQ-mamS</i> (2.5 kb)	<i>mamT-mamU</i> (1.4 kb)
1	—	—	+	—	—	—
3	—	—	+	—	—	+
4	—	+	+	—	—	—
6	—	+	+	—	—	+
7	+	—	+	+	+	+
8	0	+	+	+	+	+
9	—	+	+	—	—	+
10	+	0	+	+	+	+
11	—	+	+	+	+	+
12	+	0	+	+	+	+
13	+	+	+	+	+	+
14	+	+	+	+	+	+
15	0	+	+	—	+	+
16	+	+	+	+	+	+
19	+	+	+	+	+	+
20	+	+	+	+	+	+
22	+	+	+	+	+	+
23	+	+	+	+	+	+

^a Six regions were probed by PCR. The presence of bands was detected by agarose gel electrophoresis (see Fig. S2 in the supplemental material). —, no product detectable; +, product detectable; 0, product of aberrant size detectable.

the first *recA* mutant of a magnetotactic bacterium. Deletion of *recA* had only moderate effects on the growth rates of *M. gryphiswaldense* under aerobic conditions, indicating that *recA* is not essential for growth and magnetosome formation. This is in contrast to other bacteria like *Streptomyces lividans*, where difficulties due to essentiality in generating a full-length *recA* deletion have been suspected (23). As previously described for *E. coli*, deletions of *recA* resulted in growth defects and decreased viability (6). Under aerobic but not microaerobic and anaerobic conditions, exponential growth of *M. gryphiswaldense* IK-1 was delayed. A similar effect was also described for a *Lactococcus lactis* *recA* mutant and attributed to damage by hydroxyl radicals (OH^-) produced in the Fenton reaction during aerobic growth (10). In *M. gryphiswaldense*, a growth delay under aerobic conditions thus may also result from the inability to induce DNA repair mechanisms after damage via reactive oxygen species.

Although decreased C_{mag} values were measured for the mutant, a comparison of the size and number of magnetosomes and the intracellular iron content revealed no differences from the WT, which argues against a direct effect on magnetosome formation. Instead, the observed changes in cell dimensions and shapes likely account for the reduced magnetic response of the mutant. Measurement of the C_{mag} value is based on an optical method whereby cells are aligned parallel to the lines in a magnetic field, resulting in a change in light scattering (36). Therefore, slight deviations in cell shape might lead to differences in absorbance. An effect on cell shape and size (i.e., an increased proportion of elongated cells) was reported in a *recA*-deficient *Leptospira biflexa* strain, which was attributed to DNA segregation problems (39) and may also explain the phenotype of IK-1.

The survival rate of the IK-1 mutant strain was 28-fold lower than that of the WT, suggesting a role for the *recA* gene in stress response activation in *M. gryphiswaldense*. After transfer of the *recA* gene, partial restoration of UV sensitivity was observed. Nevertheless, the transcomplemented mutant still showed a survival rate lower than that of the WT. However, an intermediate rate of transcomplementation was also shown for other genes of *M. gryphiswaldense* (31, 46). Most notably, homologous recombination was decreased 80-fold in IK-1. Compared to other recombination-deficient mutants, such as *Rhodospirillum rubrum* (7), *Agrobacterium tumefaciens* (11), or *E. coli* (8), where the difference in recombination efficiency was at least 10^3 , the 80-fold decrease in *M. gryphiswaldense* is relatively small. However, even in the WT, the activity of RecA seems to be relatively low, as the observed frequencies of plasmid insertion were also significantly lower (2.7×10^{-7}) than those reported for other bacteria, e.g., 10^{-5} to 10^{-6} in *E. coli* (45).

Very few Km^r clones (4 transconjugants out of 1.2×10^9 recipients) were observed after conjugation of the suicide plasmids into the *recA* mutant strain. These mutants might have arisen by genomic insertion via RecA-independent recombination events, which are known to occur at low frequencies and are less responsive to the extent of homology (9). Transcomplementation of the mutant strain resulted in a frequency of homologous recombination slightly higher than that of the WT (WT, 2.7×10^{-7} ; transcomplemented mutant, 3×10^{-7}). As allelic replacement in MTB has remained tedious

due to poor recombination efficiency, transient overexpression of the *recA* gene thus might provide a way to enforce the construction of mutants in future approaches.

It was shown in previous studies that spontaneous MAI mutants affected in magnetosome formation might occur at a high frequency upon subcultivation and storage in the laboratory (32, 40). The mutations were polymorphic with respect to the sites and extents of deletions, but all mutations were found to be associated with the loss of various copies of insertion elements, most of which correspond to similar copies of transposase genes. In *M. gryphiswaldense*, 42 transposase genes were identified, which indicates that the MAI has been a genomic hot spot for multiple transposition events, which may account for its genetic instability (15). The rearrangements and deletions within the MAI hamper the analysis of magnetosome formation and may obscure genetic analysis results. A mechanism was postulated in which deletions are caused by homologous recombination between two identical insertion sequence repeat copies by a RecA-dependent mechanism. The incubation scheme used proved to be more efficient than previous approaches (10^{-2}) (40) in yielding a range of Mag^- phenotypes, which could be explained by the combination of different physiological stress conditions (temperature, starvation during stationary growth, and oxidative stress). Similar approaches were used before to isolate spontaneous pleiotropic mutants of several *Pseudomonas* species after stationary growth in nutrient-rich liquid medium for several days and correlate with a selective advantage for the cells (5, 12). We found up to 22% of the WT colonies to have pleiotropic magnetosome phenotypes, with 61% of the Mag^- clones harboring gene deletions. Furthermore, from 4 clones (8, 10, 12, and 15), PCR products with sizes differing from those expected were obtained, which might be due to rearrangement events within parts of the *mamAB* operon. Nevertheless, in 7 Mag^- mutants, no modifications within the *mamAB* operon could be detected via PCR. The residual Mag^- clones might be due to point mutations or rearrangements in genes or outside the *mamAB* operon. In contrast, we were unable to identify any white colonies among the >10,000 visually screened clones of the *recA* mutant strain. This supports the conclusion that the observed genetic instability within the MAI is, in fact, due to the activity of the RecA protein. Its significantly increased genetic stability in the absence of RecA also makes strain IK-1 a promising tool for the expression of genes and further genetic engineering, as well as the biotechnological production of bacterial magnetosomes.

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