Biosynthesis of magnetic nanostructures in a foreign organism by transfer of bacterial magnetosome gene clusters

Table S1: Summary of magnetic responses ("C_{mag}"), intracellular iron content and crystal size and number of various strains (median values, ± = standard deviation). If not indicated otherwise, cells were grown in the presence of 50 µM ferric citrate. Magnetic response and total iron content measurements were performed with (n) biological replicates under identical conditions (see also material & methods). For determination of crystal size and number per cell, cells of one clone were analyzed by TEM (n=sample size). The Mann-Whitney test (http://elegans.som.vcu.edu/~leon/stats/utest.html) was performed for crystal size comparison of *R. rubrum_ABG6X* and *R. rubrum_ABG6X_feo*: the difference was highly significant (p<0.001, two tailed test). Crystal size comparison of *R. rubrum_ABG6X_feo* and *M. gryphiswaldense* revealed no significant difference (p≥0.05, two tailed test).

Strain	Magnetic response ("C_{mag}")	Iron content (% dry weight)	Crystal size (nm)	Crystal number per cell
M. gryphiswaldense MSR-1	1.4 ± 0.2 (n=3)	3.5 (n=3)	36 ± 9 (n=310)	24 ± 8 (n=52)
M. gryphiswaldense Δ*mamAB_AB*	1.2 ± 0.2 (n=3)	n.d.	37 ± 10 (n=112)	23 ± 7 (n=24)
M. gryphiswaldense MSR-1B_*AB*	0.2 (n=3)	n.d.	17 ± 6 (n=112)	16 ± 6 (n=20)
M. gryphiswaldense MSR-1B_*ABG*	0.6 ± 0.1, (n=3)	n.d.	25 ± 6 (n=104)	13 ± 6 (n=20)
M. gryphiswaldense MSR-1B_*ABG6*	0.9± 0.2 (n=3)	n.d.	35 ± 8 (n=103)	18 ± 8 (n=22)
R. rubrum ATCC 11170	-	0.07 ± 0.04 (n=3)	-	-
R. rubrum_AB	-	0.08 (n=3)	-	-
R. rubrum_ABG	-	0.10 ± 0.01 (n=3)	-	-
R. rubrum_ABG6	-	0.17 (n=4)	12 ± 6 (n=304)	26 ± 10 (n=50)
R. rubrum_ABG6X	0.3 ± 0.2 (n=3)	0.17 ± 0.02 (n=4)	24 ± 7 (n=307)	10 ± 4 (n=50)
R. rubrum_ABG6X 500 µM ferric citrate	0.3 (n=4)	n. d.	25 ± 7 (n=301)	11 ± 5 (n=51)
R. rubrum_ABG6X 100 µM ferrous sulfate	0.2 (n=4)	n.d.	24 ± 8 (n=312)	10 ± 5 (n=52)
R. rubrum_ABG6X_ftsZm	0.6 ± 0.1* (n=3)	0.18 ± 0.03 (n=3)	26 ± 9 (n=300)	11 ± 4 (n=51)
R. rubrum_ABG6X_dJ	0.2 (n=3)	0.18 ± 0.01 (n=3)	27 ± 9 (n=300)	9 ± 4 (n=50)**
R. rubrum_ABG6X_dI	-	0.09 ± 0.07	-	-

		(n=3)		
R. rubrum_ABG6X_feo	0.8 ± 0.1 (n=3)	0.28 ± 0.07 (n=3)	37 ± 10 (n=300)	10 ± 4 (n=52)

*The slightly increased C_{mag} is likely due to effects of the genuine cell division protein FtsZm on cell morphology, as no difference in iron content and crystal size or number per cell was detectable.

**64% of mutant cells (n=32) harbored clustered magnetosomes, whereas 36% still showed a chain-like alignment of magnetosomes (n=18).

Table S2: Magnetosome proteins identified in the MM of strain *R. rubrum_ABG6X* by nano-electrospray ionization-LC tandem MS (ESI-LC-MS/MS). Spectra were analyzed via Mascot™ software using the NCBI nr Protein Database and a database from *M. gryphiswaldense*[1] (asterisks). Proteins are listed in the order of their exponentially modified protein abundance index (emPAI). The data have been deposited to ProteomeXchange with identifier PXD000348 (DOI 10.6019/PXD000348).

Protein	Accession number	Coverage (%)	No. of spectrum matches	No. of sequence peptides	Molecular weight (kDa)	Calculated pI	emPAI	Putative function
MamK	MGR_4093	57	9	9	39.6	5.4	1.51	Magnetosome chain assembly/positioning
MamC	MGR_4078	32	4	3	12.4	5.1	1.01	Crystal size and shape control [4]
MamJ	MGR_4092	32	10	6	48.6	4.0	0.76	Magnetosome chain assembly [5]
MamA	MGR_4099	37	1	1	23.9	5.7	0.65	TPR-like protein associated with the magnetosome membrane
MamF	MGR_4076	17	1	1	12.4	9.1	0.60	Magnetosome size and shape control [4]
Mms6	MGR_4073	19	1	1	12.7	9.5	0.58	Magnetosome crystallization
MamD	MGR_4077	20	3	3	30.2	9.8	0.49	Crystal size and shape control [4]
MamM*	MGR_4095	15	3	3	34.7	5.8	0.42	Iron transport/MM assembly [10]
MmsF*	MGR_4072	8	2	1	13.9	9.3	0.23	Crystal size and shape control [11]
MamB*	MGR_4102	7	1	1	32.1	5.4	0.21	Iron transport/MM assembly [10]
MamY*	MGR_4150	18	2	2	40.9	4.8	0.16	Tubulation and magnetosome membrane formation [12]
MamO*	MGR_4097	6	3	3	65.3	6.5	0.15	Magnetosome crystallization
MamE	MGR_4091	4	2	2	78.3	8.1	0.08	Magnetosome crystallization

Table S3: Strains and plasmids used in this study. Km^R= kanamycin resistance, Tc^R= tetracycline resistance, Ap^R= ampicillin resistance, BSD^R= blasticidin S resistance, Cm^R= chloramphenicol resistance, Gm^R= gentamicin resistance, $Spec^R$= spectinomycin resistance.

Strain or plasmid	Characteristics	Reference(s) or source
Magnetospirillum gryphiswaldense strains		
M. gryphiswaldense MSR-1	Wild-type (wt)	DSM-6361,[15]
M. gryphiswaldense MSR-1B	spontaneous unmagnetic mutant lacking parts of the MAI	[16]
M. gryphiswaldense Δ*mamAB*	*mamAB* deletion mutant	[17]
M. gryphiswaldense Δ*mamAB* _*AB*	Km^R, transposon mutant with inserted *mamAB* operon	This study
M. gryphiswaldense MSR-1B_*AB*	Km^R, transposon mutant with inserted *mamAB* operon	This study
M. gryphiswaldense MSR-1B_*ABG*	Km^R, $Spec^R$, transposon mutant with inserted *mamAB and mamGFDC* operon	This study
M. gryphiswaldense MSR-1B_*ABG6*	Km^R, Cm^R, transposon mutant with inserted *mamAB, mamGFDC* and *mms6* operon	This study
Rhodospirillum rubrum strains		
R. rubrum ATCC 11170	wt	[18] (kindly provided by H. Grammel, Magdeburg)
*R. rubrum*_*AB*	Km^R, transposon mutant with inserted *mamAB* operon	This study
*R. rubrum*_*ABG*	Km^R, $Spec^R$, transposon mutant with inserted *mamAB and mamGFDC* operon	This study
R. rubrum _*ABG6*	Km^R, Cm^R, transposon mutant with inserted *mamAB, mamGFDC* and *mms6* operon	This study
*R. rubrum*_*ABG6X*	Km^R, Cm^R, Gm^R transposon mutant with inserted *mamAB, mamGFDC, mms6* and *mamXY* operon (without *ftsZm*)	This study
*R. rubrum*_*ABG6X_dJ*	Km^R, Cm^R, Gm^R, Ap^R transposon mutant with inserted *mamAB* (*mamJ* deletion)*, mamGFDC, mms6* and *mamXY* operon (without *ftsZm*)	This study
*R. rubrum*_*ABG6X_dI*	Km^R, Cm^R, Gm^R, Ap^R transposon mutant with inserted *mamAB* (*mamI* deletion)*, mamGFDC, mms6* and *mamXY* operon (without *ftsZm*)	This study
*R. rubrum*_*ABG6X_ftsZm*	Km^R, Cm^R, Gm^R, Tc^R transposon mutant with inserted *mamAB, mamGFDC, mms6* and *mamXY* operon (without *ftsZm*) and *ftsZm*	This study

	under control of an inducible lac promoter	
R. rubrum_ABG6X_feo	Km^R, Cm^R, Gm^R, Tc^R transposon mutant with inserted with inserted *mamAB, mamGFDC, mms6, mamXY* and *feoAB1* operon	This study
R.rubrum_GFDC-EGFP	Tc^R transposon mutant with inserted *mamGFDC-EGFP*	This study
R.rubrum_ABG6X_GFDC-EGFP	Km^R, Cm^R, Gm^R, Tc^R transposon mutant with inserted *mamAB, mamGFDC, mms6* and *mamXY* operon (without *ftsZm*) and *mamGFDC-EGFP*	This study
R. rubrum_J-EGFP	Tc^R transposon mutant with inserted *mamGFDC-EGFP*	This study
R. rubrum_ABG6X_J-EGFP	Km^R, Cm^R, Gm^R, Tc^R transposon mutant with inserted *mamAB, mamGFDC, mms6* and *mamXY* operon (without *ftsZm*) and *mamJ-EGFP*	This study
Escherichia coli strains		
DH10b	*F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara leu) 7697 galU galK rpsL nupG λ–*	Invitrogen
BW29427	*dap* auxotroph derivative of *E. coli* strain B2155	K. Datsenko and B. L. Wanner, unpublished
WM3064	*thrB1004 pro thi rpsL hsdS lacZΔM15 RP4-1360 Δ(araBAD)567 ΔdapA1341:: [erm pir]*	W. Metcalf, kindly provided by J. Gescher, KIT Karlsruhe
Plasmids		
pSC101-BAD-gbaA	Tc^R, replicative plasmid containing redα/redβ recombinases under the control of a L-Arabinose inducible promoter, temperature sensitive origin of replication	[19]
p15A-Tps-oriT-Km	Km^R, BSD^R, oriT, p15A origin of replication, mariner tps, cloning cassette	[20]
pSSK18 (BAC_*mamAB*)	BAC containing the *mamAB* operon from *M. gryphiswaldense*	[16]
pTps_AB	Km^R, BSD^R, mariner tps vector containing *mamAB* operon	This study
pTps_ABG	$Spec^R$, Km^R, BSD^R, mariner tps vector with *mamAB* and *mamGFDC* operon	This study
pTps_ABG6	Cm^R, Km^R, BSD^R, mariner tps vector with *mamAB*, *mamGFDC*, and *mms6* operon	This study
pTps_XYZ	Gm^R, BSD^R, mariner Tps vector with *mamY*, *mamX* and *mamZ*	This study

pTps_ABG6_dJ	Cm^R, Km^R, BSD^R, Ap^R, mariner tps vector with *mamAB*, *mamGFDC*, and *mms6* operon, (*mamJ* deletion)	This study
pTps_ABG6_dI	Cm^R, Km^R, BSD^R, Ap^R, mariner tps vector with *mamAB*, *mamGFDC*, and *mms6* operon, (*mamI* deletion)	This study
pBAM1	Km^R, Ap^R, γR6K origin of replication, oriT, Tn5 vector	[21]
Tet-pBAM1	Tc^R, Ap^R, γR6K origin of replication, oriT, Tn5 vector	This study
Tet-pBam_mamGFDC-EGFP	Tc^R, Ap^R, *mamGFDC* operon under control of $P_{mamDC}$with a C-terminal EGFP fusion, Tn5 vector	This study
Tet-pBam_MamJ-EGFP	Tc^R, Ap^R, *mamJ* under control of P_{mamDC} with a C-terminal EGFP fusion, Tn5 vector	This study
pRU-1feoAB	Km^R, broad host range pBBRMCS2, *feoAB1* operon under the control of P_{mamH}	R. Uebe, unpublished
Tet-pBam_feoAB1	Tc^R, Ap^R, *feoAB1* operon under the control of P_{mamH}, Tn5 vector	This study
Tet-pBam-ftsZm_mCherry	Tc^R, Ap^R, *ftsZm, lacI* with a C-terminal mCherry fusion under control of inducible P_{lac}, Tn5 vector	This study
pFM211	Km^R, broad host range pBBRMCS2, lacI, *ftsZm* with C-terminal *mCherry* fusion, *mamK* with N-terminal EGFP fusion	F. Müller, unpublished

Table S4: Oligonucleotides used in this study.

Primer	Nucleotide sequence (5'-3')[a]	Product
Mam-tps5	AATTCGCACGGACTATAGCAACGAATCGAGGTCGGTTGAC AAGCCATAAATCAGAAGAACTCGTCAAGAAGGC	p15A-Tps-oriT-Km, ET-recombination with BAC_*mamAB,* pTps_AB
Mam-tps3	GAACGAAGATGAGACAGAAATCCGTGGCGCCGAGCGTAA GCATCCGGTGAGAACCTCATTCCCTCATGATACAG	
mamGFC3	TATCATGAGGGAATGAGGTTCTCACCGGATGCTTACGCTC GGCGCCAGAGCACATCGGGGTGAATGACGAC	*mamGFDC* operon, ET-recombination with pTps_AB
mamGFC5	CGCTAGCTGCGGGTTATTCGCATTTGC	
spectMam3	TCAAAACCCGCGCAGAGGCAAATGCGAATAACCCGCAGCT AGCGTTATAATTTTTTTAATCTGTTATT	Spectinomycin resistance cassette, ET-recombination with pTps_AB
spectMam5	TGATCCGCTATGGTAAGCGCATCATGTCCGGATCCCATGG CGTTCCGCTCGTAACGTGACTGGCAAGAGATATT	
mms6cm5	TACTGCGATGAGTGGCAGGGCGGGGCGTAAGCTTACAATT TCCATTCGCCATTC	*mms6* operon, ET-recombination with pTps_ABG
mms6mam3	GTGCTTCGCTGTGTCCACAAGAACC	
cm-mms6-3	TGGCGAATGGAAATTGTAAGCTTACGCCCCGCCCTGCCAC TC	Chloramphenicol resistance cassette, ET-recombination with pTps_ABG
cm-mms6-5	TGATCCGCTATGGTAAGCGCATCATGTCCGGATCCCATGG CGTTCCGCTCGTCCTGGTGTCCCTGTTGATACC	
IK097	TCTAGAGGGCCCCAACTTTTTCGCTTTACTAGCTCTTAGTT CTCCAATAAATTCCCTGCGTCGA	P_{mamDC} in pBAM1
IK098	CATATGCTGATCTCCGGCAAGTGTATGCACGATTCCCTCTC TGCCCCTTAAAATCGACGCAGGGAAT	
IK107	CATATGATCAAGGGCATCGCGGG	*mamGFDC* operon in pBAM1
IK101	GGTACCGGCCAATTCTTCCCTCAGAA	
IK102	GGTACCGGAGGCGGAGGCGGT	*egfp* in pBAM1
IK103	GAATTCTTACTTGTACAGCTCGTCCATG	
IK163	GAATTCTTAGCCGATTCGCAG	*mamXY*-operon (without ftsZm), ET-recombination with p15A-Tps-oriT-Gm
IK164	GAGCTCGGCAGCCTCATTTAAA	
IK173	CCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGG AAGCCCTGCAACGTATAATATTTGCCCATG	Gentamicin resistance cassette, ET-Recombination with p15A-Tps-oriT-Km
IK174	AGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCG ATACCGTAAAGCGATCTCGGCTTGAA	
IK208	CCCGGTACCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTG CGCGCTTGGCCTCATTCCCTCATGATACAGAGAC	p15A-Tps-oriT-Gm, ET-recombination with *mamXY*
IK209	GGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTT CGCCAGCTGTCTCGGCTTGAACGAATTG	
IK213	GACGTCGAGCCACGGCGG	Tetracycline resistance cassette in pBAM1
IK214	GGGTCCCTCAGGTCGAGGTGGC	
IK215	TCTAGACTACAAGAATGTCCCGC	*feoAB1* operon+P_{mamH} in pBAM1
IK216	GAATTCGGCATCCTGATCGGT	
IK217	CATATGATGGCAAAAAACCGG	*mamJ* in pBAM1
IK218	GGCGGTACCTTTATTCTTATCTTCAGCATCAC	
IK235	GGGTGGAGCGGGATAATGGCAAAAAACCGGCGTGATCGC GGCACGGCTAAATACATTCAAATATGTATCC	Ampicillin resistance cassette insertion into *mamJ* of pTps_ABG6
IK236	CTATTTATTCTTATCTTCAGCATCACATTTCGGCGATGAACA ACTACCTTACCAATGCTTAATCAGTG	
IK239	CGCCGCTTGTGTTCTGTATCAAGACTGGAGAACGTTTATG CCAACTAAATACATTCAAATATGTATCC	Ampicillin resistance cassette Insertion into *mamI* of pTps_ABG6
IK240	TCAACCATCGATGTTAGGGTCTGAGTTCGCCCTCTTACCG GCAGGTTACCAATGCTTAATCAGTG	
IK251	AAACCGCCCAGTCTAGCTATCGCCATGTAAGCCCACTGCA AGCTACCTGCCCTCATTCCCTCATGATACA	Tet-pBAM1, ET-recombination with

IK252	CAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGA GGCCCGCACCGGATTTTGAGACACAAGACGTC	recombination with pFM211

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