Supplementary Tables

Strains and plasmids	Description	References
M. gryphiswaldense **strains**		
MSR-1 R3/S1	Rif^R Sm^R, spontaneous mutant	(1)
MSR-1B	R3/S1 Spontaneous mutant, lacking 40,385 kb genomic region	(2)
IK-1	R3/S1 Δ*recA*	(3)
2xGFDC**op**	one native and one further copy of the *mamGFDC* operon, Km^R	This study
2x*mms6*op	one native and one further copy of the *mms6* operon, Km^R	This study
2x*mms6*op+2x*GFDC*	one native and one further copy of the *mms6* and *mamGFDC* operon, Km^R	This study
3x*mms6*op	one native and two further copies of the *mms6* operon, Km^R	This study
4x*mms6*op/Gm	one native and three further copies of the *mms6* operon, Km^R, Gm^R	This study

4x*mms6*op	one native and three further copies of the *mms6* operon, Km^R, Gm^S	This study
5x*mms6*op	one native and five copies of the *mms6* operon, Km^R, Gm^S	This study
2x*mamAB*op	one native and one further copy of the *mamAB* operon, Km^R	This study
3x*mamAB*op	one native and two copies of the *mamAB* operon, Km^R, Gm^R	This study
2x*ABG6X*	ΔRecA with one further copy of the *mamAB*, *mamGFDC*, *mms6* operon and *mamXYZ* genes	This study
ABG6X*+*feo	ΔRecA with two copies of the *mamAB*, *mamGFDC*, *mms6* operon and *mamXYZ* genes and *feoAB1* operon	This study
***E. coli* strain**		
BW29427	*thrB1004 pro thi rpsL hsdS lacZDM15 RP4-1360D(araBAD)567DdapA*::[*erm pir*]	Datsenko and Wanner (unpublished)
DH5α	*1341::[erm*	Invitrogen

	pir(wildtype)]trahsdR17 recA1-endA1gyrA96thi-1relA1	
DH10β	*F⁻ endA1 recA1 galE15 galK16 nupG rpsL ΔlacX74 Φ80lacZΔM15 araD139 Δ(ara,leu)7697 mcrA Δ(mrr-hsdRMS-mcrBC) λ⁻*	Invitrogen
S17-1*λpir*	RPA-2, Tc::Mu-Km::Tn7 (λpir)	(4)
WM3064	*thrB1004 pro thi rpsL hsdS lacZDM15 RP4-1360D(araBAD) 567DdapA*::[*erm pir*]	W. Metcalf, kindly provided by J. Gescher, KIT Karlsruhe
Plasmids		
pJet1.2	Ap^r, *eco47IR*, *rep* (pMB-1)	Fermentas
pCM157	Tet^R, Cre expression vector	(5)
pBBR-MCS5	Gm^r, *lacZa*	(2)
pBam-1	*ori*R6K, Km^R, Ap^R	(6)
pTps_AB	Km^R, BSD^R, mariner tps vector containing *mamAB* operon	(7)
Gm- pTps_AB	Gm^R, BSD^R, mariner tps vector containing *mamAB* operon	This study

pTps_ABG6	Cm^R, Km^R, BSD^R, mariner tps vector with *mamAB*, *mamGFDC*, and *mms6* operon	(7)
pTps_XYZ	Gm^R, BSD^R, mariner Tps vector with *mamY*, *mamX* and *mamZ*	(7)
Tet-pBam_feoAB1	Tc^R, Ap^R, *feoAB1* operon under the control of P_{mamH}, Tn5 vector	(7)
pBam_*mamGFDC*	pBam-1, *mamGFDC* operon	This study
pBam_*mms6* 1x	pBam-1, *mms6* operon	This study
pBam_GFDC/*mms6*	pBam_*mamGFDC*, *mms6* operon	This study
pBam_*mms6* 2x	pBam_*mms6* 1x , *mms6* operon (two *mms6* operons)	This study
pBam_GFDC/*Gm*	pBam_*mamGFDC,* *lox71*_Gm^r_*lox66*	This study
pBam_*mms6*/Gm	pBam_*mms6* 1x, *lox71*_Gm^r_*lox66*	This study

Supplementary Table S1: Strains and plasmids used in this study.

Strain	Revealed insertion site/s of operon copies (gene name)	Predicted name or function of the encoded protein
***2xGFDC*op**	*amb3829* homolog	Flagellar protein FlbT
2x*mms6*op	*mgr0892*	Hemolysin activation/secretion protein
2x*mms6op*+2x*GFDC*	*mgr2596*	Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase
3x*mms6*op	*mgr1078*	AMP fatty acid ligase
4x*mms6*op	*mgr1078*	AMP fatty acid ligase;
	mgr3739	TerC-like integral membrane protein
***5xmms6*op**	*mgr1078*	AMP fatty acid ligase;
	mgr3739	TerC-like integral membrane protein;
	mgr1637	Methyl-accepting chemotaxis protein
2x*mamAB*op	*mgr2709*	molecular chaperone distantly related to HSP70-fold metalloproteases
3x*mamAB*op	*mgr2709*	Molecular chaperone distantly related to HSP70-fold metalloproteases;
	mgr3096	NADPH-dependent glutamate synthase beta

		chain and related oxidoreductases
2x*ABG6Xop*	*mgr2922*	Hypothetical protein;
	mgr0484	Hypothetical protein
2x*ABG6Xop+2xfeo*	*mgr2922*	Hypothetical protein;
	mgr0484	Hypothetical protein;
	mgr2581	Membrane or secreted protein

Supplementary Table S2: Transposon insertion sites in generated overexpression strains identified by genome sequencing.

Supplementary Figures

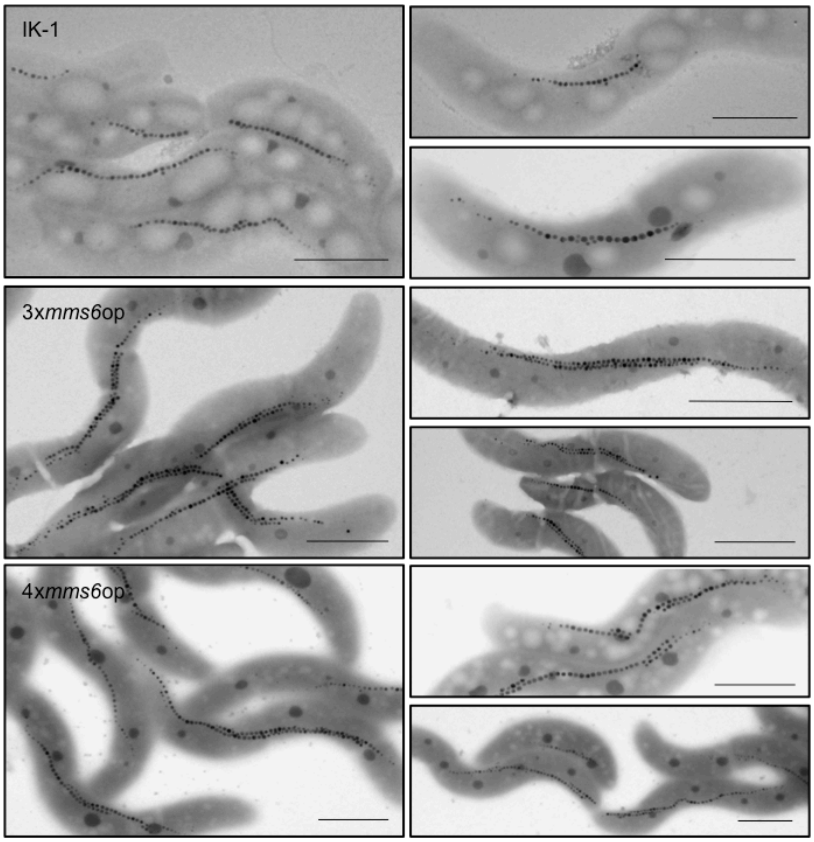

Figure S1. Transmission electron micrographs of mutants 3x*mms6*op and 4x*mms6*op compared to IK-1. Scale bar = 1 μm.

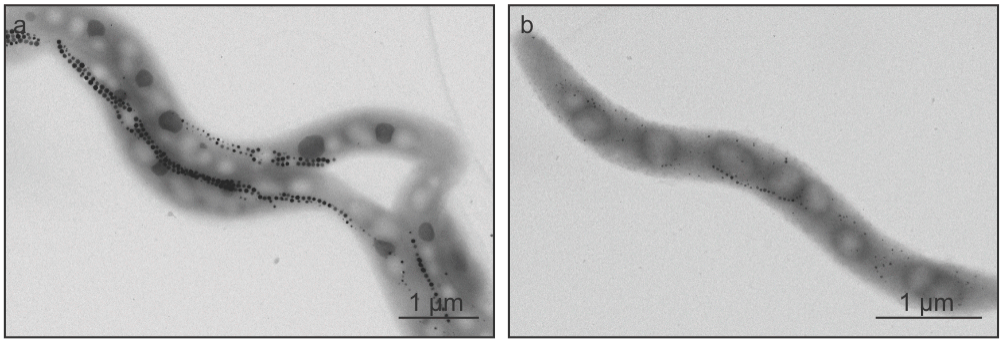

Figure S2. Transmission electron micrographs of 2x*mamAB*op, illustrating the phenotypic heterogeneity of magnetosome formation found in this mutant. (**a**) Cell with increased numbers of regular-sized magnetosomes. (**b**) Cell with increased number of magnetosomes with aberrant crystal sizes and intracellular localization.

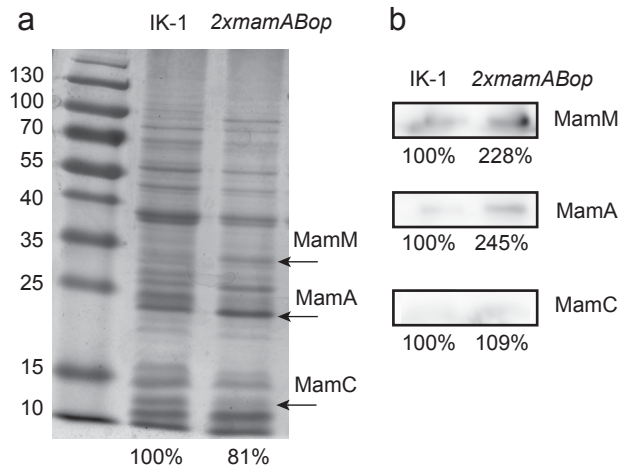

Figure S3. Proteomic analysis of magnetosomes from 2x*mamAB*op. (**a**) 1D SDS-PAGE of Coomassie blue stained proteins, which were solubilized from isolated magnetosome particles of IK-1 and 2x*mamAB*op. Bands of size of MamM (34 kDa), MamA (24 kDa) and MamC (12 kDa) are indicated (arrows). (**b**) Immnuodetection of MamM, MamA and MamC in blotted magnetosome membrane fractions from IK-1 and 2x*mamAB*op. Respective protein bands of strain IK-1 were assigned to 100%.

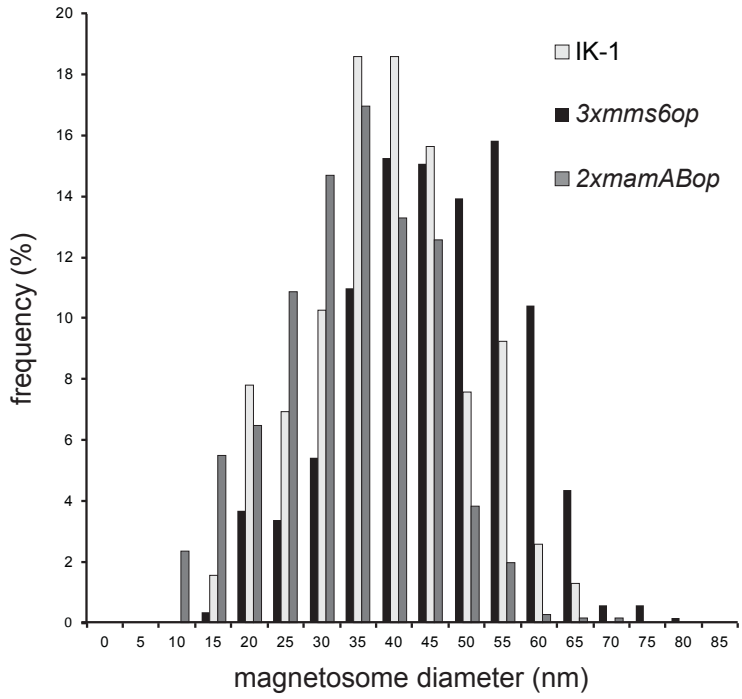

Figure S4. Magnetosome size distributions of the insertion mutant 3x*mms6*op, 2x*ABG6X*op and the parental strain IK-1.

Supplementary References

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5. **Marx CJ, Lidstrom ME.** 2002. Broad-host-range cre-lox system for antibiotic marker recycling in gram-negative bacteria. Biotechniques **33:**1062-1067.
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7. **Kolinko I, Lohsse A, Borg S, Raschdorf O, Jogler C, Tu Q, Posfai M, Tompa E, Plitzko JM, Brachmann A, Wanner G, Müller R, Zhang Y, Schüler D.** 2014. Biosynthesis of magnetic nanostructures in a foreign

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