Supplemental materials:

The periplasmic nitrate reductase Nap is required for anaerobic growth and involved in redox control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*

Running title: Denitrification and magnetite biomineralization

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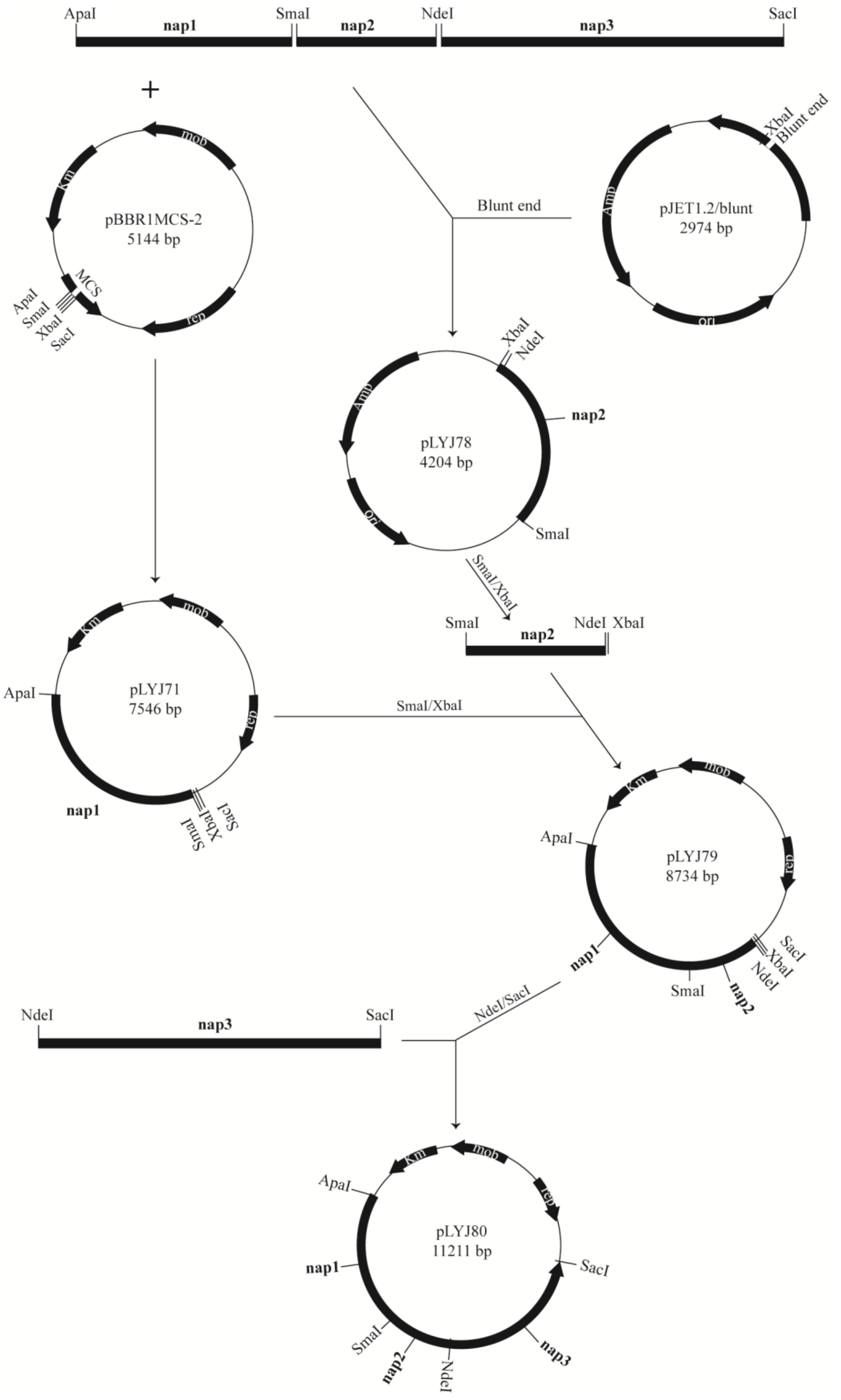

Fig. S1. Scheme for the construction of the large complementation plasmid pLYJ80 (about 11 kb) for Δ*nap*.

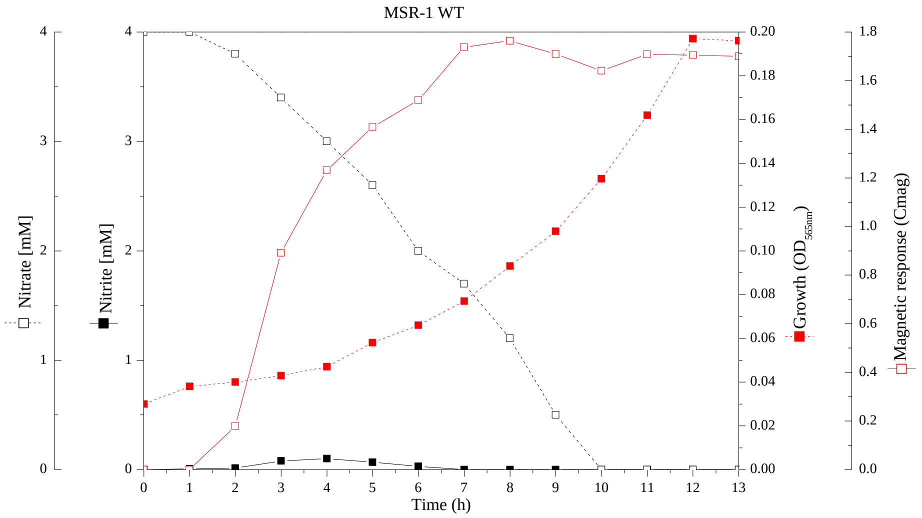

Fig. S2. Nitrate utilization in WT with an initial nitrate concentration of 4 mM (nitrate medium).

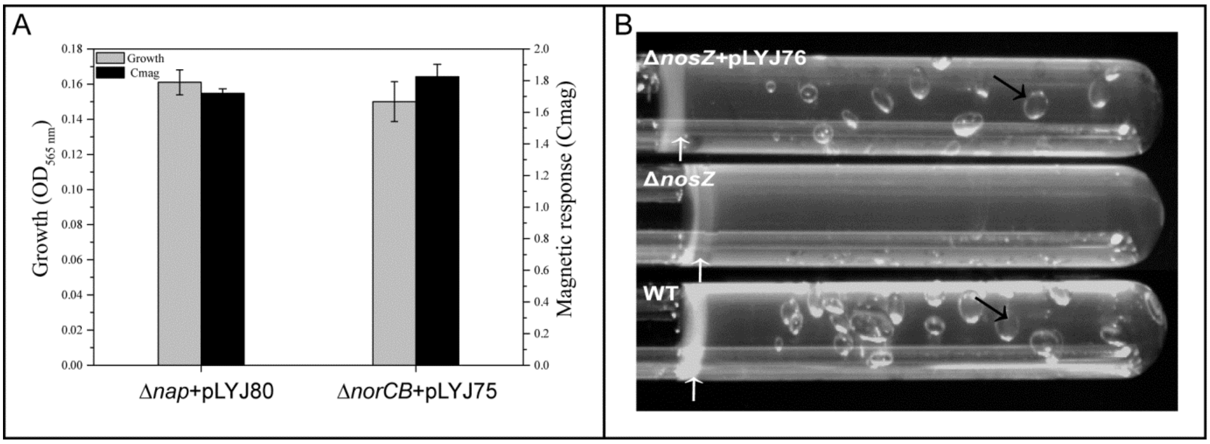

Fig. S3. (A) Growth ($OD_{565\ nm}$) and magnetic response (Cmag) of Δ*nap*+pLYJ80 and Δ*norCB*+pLYJ75 under anaerobic conditions. Δ*nap*+pLYJ80 and Δ*norCB*+pLYJ75 represented mutant cells harboring their respective WT alleles. (B) Gas production assay in WT, Δ*nosZ* and Δ*nosZ*+pLYJ76 in semisolid agar. Δ*nosZ*+pLYJ76 represented *nosZ* mutant cell harboring a WT *nosZ* allele. Cells grew as bands (white arrows), and bubbles (black arrows) were detected in WT and complemented *nosZ* strain cultures but not in Δ*nosZ* mutant.

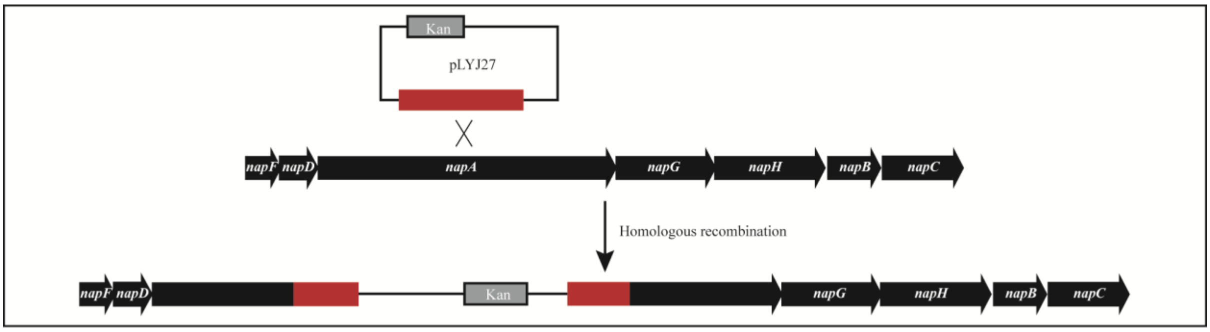

Fig. S4. Schematic representation of steps employed for the interruption of *napA* (*napA::kanR*) in MSR-1. The insertion mutant showed WT-like growth and Cmag values. Under microaerobic and anaerobic conditions, it still consumed nitrate like the WT (data not shown).

Table S1. Bacterial strains used in this work

Strain	Important feature (s)	Source or reference
E. coli strain DH5α	F’ Φ80d*lacZ*ΔM15Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsd*R17 (r_k-, m_k+) *pho*A *sup*E44 λ- *thi*-1 *gyr*A96 *rel*A1	Invitrogen
E. coli strain BW29427	*dap* auxotroph derivative of *E. coli* strain B2155	K. Datsenko and B. L. Wanner, unpublished
MSR-1 WT	Wild type R3/S1, but Rif^r, Sm^r	(8)
Δ*nirS*Δ*norCB*	R3/S1 Δ*nirS*Δ*norCB*	Y. Li and D. Schüler, unpublished data
napA::kanR	R3/S1 *nap::pLYJ27*	This study
Δ*nap-up*	R3/S1 *nap::pLYJ85*	This study
Δ*nap-up-down*	R3/S1 *nap::pLYJ85::pLYJ92*	This study
Δ*nap*	R3/S1 Δ*nap*	This study
Δ*norCB*	R3/S1 Δ*norCB*	This study
Δ*nosZ*	R3/S1 Δ*nosZ*	This study

Table S2. Plasmids used in this work

Plasmid	Important feature (s)	Source or reference
pJTE1.2/blunt	Amp^r, *eco47IR* (lethal restriction enzyme gene), *rep* (pMB-1)	Fermentas
pCM184	Broad-host-range allelic exchange vector, Amp^r, Km^r, Tc^r	(5)
pBBR1MCS-2	Km^r, mobilizable broad-host-range vector	(3)
pK19mobGII	-	(2)
pAL01	Km^r, pK19mobGII vector (Km^r, pMB-1 replicon, *gusA*, *lacZ*) containing a 2 kb fragment upstream of *mgr4019*	(4)
pAL02/2	Gm^r, pT18mob2 vector containing a 2 kb fragment downstream of *mgr4019*	(4)
pCM157	Tc^r, Cre recombinase expression vector	(5)
pLYJ27	pCM184 plus *napA* 2-kb upstream and internal region	This study
pLYJ31	pCM184 plus *norCB* 2-kb upstream region	This study
pLYJ32	pCM184 plus *nosZ* 2-kb downstream region	This study
pLYJ34	pLYJ31 plus *norCB* 2-kb downstream region	This study
pLYJ35	pLYJ32 plus *nosZ* 2-kb upstream region	This study
pLYJ71	pBBR1MCS-2 plus *nap1* with its own promoter	This study
pLYJ75	pBBR1MCS-2 plus *norCB* with its own promoter	This study
pLYJ76	pBBR1MCS-2 plus *nosZ* with its own promoter	This study
pLYJ78	pJET1.2/blunt plus *nap2*	This study
pLYJ79	pLYJ71 plus *nap2* from pLYJ78	This study
pLYJ80	PLYJ78 plus *nap3*	This study
pLYJ85	pAL01 plus *nap* 2-kb upstream region	This study
pLYJ92	pAL02/2 plus *nap* 2-kb downstream region	This study
pLYJ97	pBBR1MCS-2 plus *gusA* from pK19mobGII	This study
pLYJ94	pLYJ97 plus *nirS* promoter region	This study
pLYJ98	pLYJ97 plus *nap* promoter region	This study
pLYJ99	pLYJ97 plus *norCB* promoter region	This study
pLYJ100	pLYJ97 plus *nosZ* promoter region	This study

Table S3. Effect of different electron acceptors and oxygen on growth and magnetic response

Culture condition	**Nitrogen source**	**Growth ($\Delta OD_{565\ nm}$[a])**	**Magnetic response (Cmag)**
Anaerobic	4 mM NO_3^-	0.18 ± 0.02	1.8 ± 0.2
	1 mM NO_2^-	0.01 ± 0.00	1.7 ± 0.0
	10 mM N_2O	0.01 ± 0.000	1.7 ± 0.0
	4 mM NH_4^+	0	ND[b]
Microaerobic	4 mM NO_3^-	0.23 ± 0.01	2.1 ± 0.2
	4 mM NH_4^+	0.17 ± 0.06	2.0 ± 0.2
Aerobic	4 mM NO_3^-	1.21 ± 0.04	0
	4 mM NH_4^+	1.25 ± 0.07	0

[a] Values represent means and standard deviations were obtained with triplicate cultures and repeated three times.

[b] Cmag value was not tested.

Table S4. BlastP analysis results of denitrification genes in MTB and non-MTB using MSR-1 as a query.

Gene in MSR-1	**Encoded gene product (aa, kDa, pI)**	**AMB-1 (e-value, similarity)**	**MS-1 (e-value, similarity)**	**MC-1 (e-value, similarity)**	**Best hit in non-MTB (e-value, similarity)**
mgr_4000*	NapF, small transmembrane protein of unknown function (7) (102, 10.17, 8.67)	*amb2692* (1e-18, 64%)[b]	*BAB59020.1* (4e-13, 67%)	*mmc1_1591* (2e-10, 62%)	*Serratia odorifera* 4Rx13 (1e-18, 64%)
mgr_4001*	NapD, cytoplasmic protein (7) (102, 11.21, 4.91)	*amb2691* (5e-43, 76%)	*magn03008202* (2e-41, 77%)	*mmc1_1592* (8e-07, 54%)	*Beggiatoa sp.* PS (9e-17, 62%)
mgr_4002*	NapA, nitrate reductase (NR) catalytic subunit containing molybdenum cofactor and a [4Fe-4S] cluster (6) (835, 93.60, 8.70)	*amb2690* (0, 91%)	*magn03008203* (0, 91%)	*mmc1_1591* (0, 83%)	*Azoarcus sp.* BH72 (0, 85%)
mgr_4003*	NapG, soluble protein (1) (272, 28.92, 7.44)	*amb2689* (2e-125, 78%)	*magn03008204* (4e-124, 79%)	*mmc1_1590* (4e-93, 71%)	*Laribacter hongkongensis* HLHK9 (3e-111, 78%)
mgr_4004*	NapH, membrane protein (1) (304, 32.72, 9.42)	*amb2688* (9e-132, 77%)	*magn03008205* (3e-125, 77%)	*mmc1_1589* (4e-85, 65%)	*Dechloromonas aromatica* RCB (3e-112, 70%)
mgr_4005*	NapB, NR subunit, a *c*-type cytochrome (6) (148, 16.44, 8.66)	*amb2687* (1e-63, 79%)	*magn03008206* (1e-66, 79%)	*mmc1_1588* (8e-31, 56%)	*Pseudovibrio sp.* JE062 (5e-38, 65%)
mgr_4006*	NapC, a membrane-bound tetraheme (6) (222, 25.53, 8.91)	*amb2686* (3e-115, 87%)	*magn03008207* (4e-114, 87%)	*mmc1_1587* (4e-77, 72%)	*Pseudovibrio sp.* JE062 (4e-90, 71%)
mgr_1052	Nitrite reductase, NirS (540, 59.26, 8.81)	*amb1395* (0, 91%) *amb4165* (0, 78%)	*magn03008451* (0, 91%)	–	*Dechlorosoma suillum* PS (0, 90%)

mgr_3484*	Nitric-oxide reductase subunit C, NorC (149, 16.23, 6.90)	*amb2945* (5e-84, 88%)	–	*mmc_0121* (4e-57, 72%)	*Nitrosococcus halophilus* Nc4 (1e-74, 81%)
mgr_3485*	Nitric-oxide reductase subunit B, NorB (445, 50.35, 8.82)	*amb2944* (0, 93%)	–	*mmc_0120* (1e-179, 73%)	*Azospirillum sp*. B510 (0, 87%)
mgr_3486	NorQ (262, 28.95, 5.40)	*amb2943* (1e-151, 90%)	–	*mmc_0119* (1e-108, 77%)	*Halomonas halodenitrificans* (1e-144, 86%)
mgr_3487	NorD (632, 70.80, 7.75)	*amb2942* (0, 78%)	–	*mmc_0117* (2e-64, 51%)	*Nitrosococcus halophilus* Nc4 (0, 70%)
mgr_2761*	Nitrous-oxide reductase, NosZ (760, 83.78, 6.06)	*amb3086* (0, 90%)	*magn03008954* (9e-145, 92%) *magn03007281* (0, 88%)	–	*Dechlorosoma suillum* PS (0, 86%)

*indicate genes deleted.

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