Supplemental materials

The terminal oxidase *cbb*$_3$ functions in redox control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*

Running title: Oxygen respiration and magnetite biomineralization

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Fig. S1 Biomineralization phenotypes of Δcbb_3 and $\Delta aa_3\Delta cbb_3$ mutants incubated for 24 h (A and C) and 35 h (B and C) in microaerobic nitrate medium. Scale bar, 500 nm.

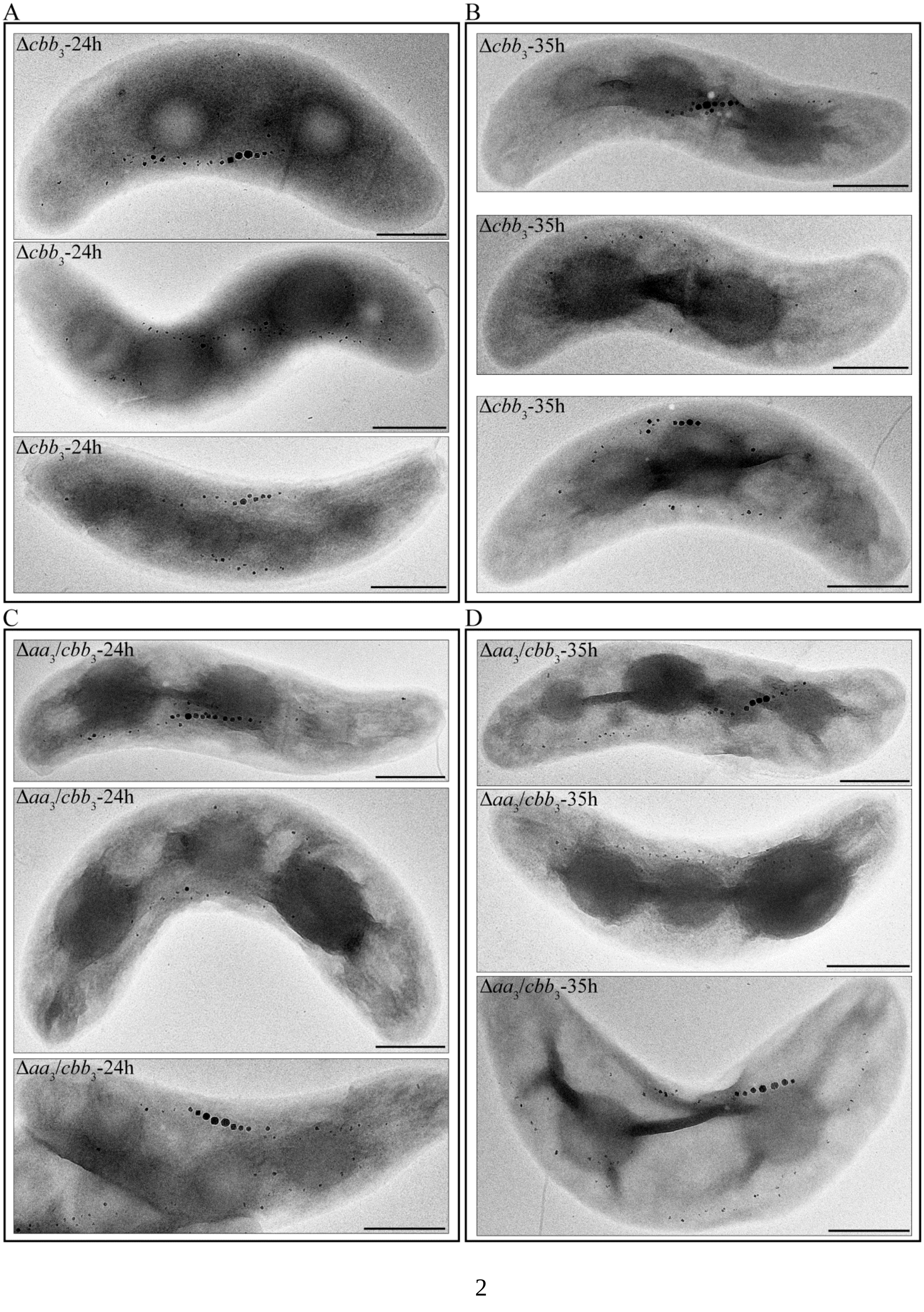

Table S1 Bacterial strains and plasmids used in this work

Strain or plasmid	Description	Reference
Strains		
E. coli 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E. coli BW29427	*dap* auxotroph derivative of *E. coli* strain B2155	K. Datsenko and B. L. Wanner, unpublished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	(1)
Δ*Mgfnr*	R3/S1, Δ*Mgfnr*	(Y. Li et al., submitted)
Δaa_3	R3/S1, Δaa_3	This study
Δ*bd*	R3/S1, Δ*bd*	This study
Δcbb_3	R3/S1, Δcbb_3	This study
$\Delta bd\Delta aa_3$	R3/S1, $\Delta bd\Delta aa_3$	This study
$\Delta aa_3\Delta cbb_3$	R3/S1, $\Delta aa_3\Delta cbb_3$	This study
$\Delta bd\Delta cbb_3$	R3/S1, $\Delta bd\Delta cbb_3$	This study
Plasmids		
pBBR1MCS-2	Km^r, mobilizable broad-host-range vector	(2)
pOR093	*mamX* CXXCH (65,104)->AXXAH, pK19mobGII derivative, Km^r	(3)
pLYJ97	pBBR1MCS-2 plus *gusA* from pK19mobGII	(4)
pLYJ115	pLYJ97 plus cbb_3 promoter region	This study
pLYJ128	pOR093 plus 2-kb fused flanking section of *ccoNOQP*	This study
pLYJ129	pOR093 plus 2-kb fused flanking section of *coxBAC*	This study
pLYJ130	pOR093 plus 2-kb fused flanking section of *cydAB*	This study
pLYJ135	pLYJ97 plus aa_3 promoter region	This study
pLYJ137	pLYJ97 plus *bd* promoter region	This study
pLYJ138	pBBR1MCS-2 plus cbb_3 with its own promoter	This study
pLYJ139	pBBR1MCS-2 plus aa_3 with its own promoter	This study
pLYJ140	pBBR1MCS-2 plus *bd* with its own promoter	This study

Table S2 BlastP analyses of operons encoding terminal oxidases in MTB and non-MTB using MSR-1 as a query.

Protein in MSR-1	Encoded gene product (aa, kDa, pI)	AMB-1 (e-value, similarity)	MS-1 (e-value, similarity)	MC-1 (e-value, similarity)	RS-1 (e-value, similarity)	Best hit in non-MTB (e-value, similarity)
mgr_2544	CcoN (532, 60.6, 9.09)	-	WP_009870373.1 (0.0, 93%)	Mmc1_2353 (0.0, 80%)	-	*Novispirillum itersonii* (0.0, 87%)
mgr_2545	CcoO (243, 27.0, 6.30)	Amb4363 (4e-137, 86%)	WP_009870372.1 (7e-137, 86%)	Mmc1_2354 (1e-94, 70%)	-	*Novispirillum itersonii* (2e-124, 82%)
mgr_2546	CcoQ (36, 4.4, 5.09)	Amb4364 (3e-09, 91%)	-	-	-	*Rhodospirillum rubrum* F11 (2e-11, 88%)
mgr_2547	CcoP (291, 31.2, 5.90)	Amb4365 (8e-133, 76%)	WP_009870371.1 (1e-134, 77%)	Mmc1_2355 (4e-52, 49%)	-	*Phaeospirillum molischianum* (5e-123, 73%)
Mgr_0911	CoxB (269, 30.1, 6.29)	Amb2170 (1e-108, 76%)	WP_009868696.1 (3e-113, 77%)	-	-	*Novispirillum itersonii* (6e-105, 77%)
Mgr_0912	CoxA (526, 57.4, 7.96)	Amb2169 (0.0, 89%)	WP_009868695.1 (0.0, 89%)	-	-	*Novispirillum itersonii* (0.0, 88%)
Mgr_0914	CoxC (264, 29.5, 6.97)	Amb2168 (2e-115, 79%)	WP_009868693.1 (8e-104, 78%)	-	-	*Caenispirillum salinarum* (7e-105, 72%)
Mgr_0697	CydB (382, 41.3, 6.06)	-	-	-	DMR_06970 (1e-63, 54%) DMR_28310 (3e-23, 43%)	*Novispirillum itersonii* (0.0, 87%)
Mgr_0698	CydA (518, 57.6, 8.66)	-	-	-	DMR_06960 (2e-61, 46%) DMR_28300 (4e-108, 55%)	*Novispirillum itersonii* (0.0, 92%)

AMB-1: *Magnetospirillum magneticum*; MS-1: *Magnetospirillum magnetotacticum*; MC-1: *Magnetococcus marinus*; RS-1: *Desulfovibrio magneticus*.The *ccoN* gene encoding the catalytic subunit of cbb_3 oxidase is not found in *M. magneticum* while the *ccoQ* gene encoding a small membrane protein of unknown function is missing in *Mc. marinus* and the incomplete genome assembly of *M. magnetotacticum*. *Mc. marinus* strain RS-1 harbors two *cydAB* operons as well as another operon *cyoABCDE* encoding a quinol oxidase bo_3 oxidase (*DMR_14870*, *_14880*, *_14890*, *_14900*, and *_14910*).

Table S3 Growth ($OD_{565\ nm}$), magnetic response (C_{mag}), and magnetosome crystal size of various terminal oxidase mutants under different conditions.

Strain	$OD_{565\ nm}$	C_{mag}	Crystal size (nm)	*n*
0% O_2				
WT	0.13 ± 0.00	1.6 ± 0.1	38.0 ± 15.8	335
Δaa_3	0.13 ± 0.00	1.7 ± 0.0	38.4 ± 14.9	335
Δbd	0.13 ± 0.00	1.6 ± 0.1	34.0 ± 18.2	427
$\Delta bd\Delta aa_3$	0.14 ± 0.02	1.7 ± 0.1	35.9 ± 24.3	304
Δcbb_3	0.14 ± 0.01	1.7 ± 0.2	35.2 ± 23.4	300
$\Delta aa_3\Delta cbb_3$	0.13 ± 0.00	1.7 ± 0.1	34.6 ± 16.6	470
$\Delta bd\Delta cbb_3$	0.14 ± 0.01	1.6 ± 0.0	38.4 ± 19.9	318
2% O_2, +NO_3^-				
WT	0.22 ± 0.01	1.6 ± 0.0	28.0 ± 15.3	337
Δaa_3	0.22 ± 0.01	1.6 ± 0.0	30.1 ± 15.0	334
Δbd	0.20 ± 0.01	1.7 ± 0.0	30.1 ± 14.6	309
$\Delta bd\Delta aa_3$	0.22 ± 0.01	1.7 ± 0.1	31.1 ± 13.0	319
2% O_2, +NH_4^+				
WT	0.11 ± 0.00	1.5 ± 0.1	-	-
Δaa_3	0.12 ± 0.01	1.5 ± 0.0	-	-
Δbd	0.10 ± 0.01	1.4 ± 0.1	-	-
$\Delta bd\Delta aa_3$	0.11 ± 0.00	1.4 ± 0.1	-	-

Number of crystals measured for each strain (*n*) is presented in the last column.

References

1. **Schultheiss D, Kube M, Schüler D.** 2004. Inactivation of the flagellin gene *flaA* in *Magnetospirillum gryphiswaldense* results in nonmagnetotactic mutants lacking flagellar filaments. Appl. Environ. Microbiol. **70:**3624-3631.
2. **Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, Peterson KM.** 1995. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene **166:**175-176.
3. **Raschdorf O, Müller FD, Posfai M, Plitzko JM, Schüler D.** 2013. The magnetosome proteins MamX, MamZ and MamH are involved in redox control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*. Mol. Microbiol. **89:**872-886.
4. **Li Y, Katzmann E, Borg S, Schüler D.** 2012. The periplasmic nitrate reductase Nap is required for anaerobic growth and involved in redox control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*. J. Bacteriol. **194:**4847-4856.