Expression of extracellular multiheme cytochromes discovered in a betaproteobacterium during Mn(III) reduction

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Running title: Novel undecaheme in Betaproteobacteria

Abstract: Soluble ligand-bound Mn(III) can support anaerobic microbial respiration in diverse aquatic environments. Thus far, Mn(III) reduction has only been associated with certain *Gammaproteobacteria*. Here, we characterized microbial communities enriched from Mn-replete sediments of Lake Matano, Indonesia. Our results provide the first evidence for biological reduction of soluble Mn(III) outside of the *Gammaproteobacteria*. Metagenome assembly and binning revealed a novel betaproteobacterium, which we designate "*Candidatus* Dechloromonas occultata." This organism dominated the enrichment and expressed a novel cytochrome c-rich protein cluster (Occ), including an undecaheme putatively involved in extracellular electron transfer during Mn(III) reduction. The *occ* gene cluster was detected in diverse aquatic bacteria, including uncultivated *Betaproteobacteria* from the deep subsurface. These observations provide new insight into the taxonomic and functional diversity of microbially-driven Mn(III) reduction in natural environments.

Main text: Manganese(III) is the most recently discovered player in the manganese cycle (10). Ligand-bound Mn(III) is often the most abundant Mn species in aquatic ecosystems (18), yet knowledge about microbes cycling Mn(III) remains fragmentary. To date, only *Shewanella* spp. (*Gammaproteobacteria*) are known to respire soluble Mn(III) using the Mtr pathway (17, 22, 23). The Mtr pathway forms a porin-cytochrome (PCC) conduit that transports electrons across the periplasm (11) for extracellular respiration of Mn(III/IV), Fe(III), and other metals (20, 23). Another PCC is Mto used by freshwater *Betaproteobacteria* for extracellular Fe(II) oxidation (7, 12, 16). Environmental omics suggests that metal reduction by *Betaproteobacteria* may be widespread in the deep subsurface (1, 13). However, only a few Fe(III)-reducing *Betaproteobacteria* isolates have been characterized (5, 8). This study presents the first evidence

for biological reduction of soluble Mn(III) by a bacterium outside of the *Gammaproteobacteria* class.

We explored microbial Mn(III) reduction in enrichments inoculated with sediment from Lake Matano, Indonesia, which has active microbial Mn and methane (CH_4) cycles (15). Manganese reduction coupled to CH_4 oxidation is a thermodynamically favorable metabolism, and its natural occurrence is supported by biological evidence (2) and geochemical evidence (4, 19). We designed an enrichment strategy to select for microbes capable of anaerobic CH_4 oxidation coupled to soluble Mn(III) reduction incubating anoxic Lake Matano with soluble Mn(III)-pyrophosphate as the electron acceptor (with 2% O_2 in a subset of bottles), and CH_4 as the sole electron donor and carbon source (see **Supplemental Material** for enrichment details). Cultures were transferred into fresh media after Mn(III) was completely reduced to Mn(II) for a total of five transfers over 395 days. By the fourth transfer, cultures with CH_4 headspace (with or without 2% O_2) reduced ~80% of soluble Mn(III) compared to ~30% with N_2 headspace (**Fig. 1**). 16S rRNA gene sequences were dominated by *Betaproteobacteria* (*Rhodocyclales*) and *Deltaproteobacteria* (*Desulfuromonadales*), and $^{13}CH_4$ oxidation to $^{13}CO_2$ was undetectable (**Figs. S1, S2**).

Samples for metagenomic and metaproteomic analysis were harvested from the fifth transfer (**Fig. 1; Fig. S1**). Out of 2,952 proteins identified in the proteome, 90% were assigned to *Betaproteobacteria*; of those, 72% mapped to a 99.53% metagenome-assembled genome (MAG; *Rhodocyclales* bacterium GT-UBC; NCBI accession QXPY01000000) with 81-82% average nucleotide identity (ANI) and phylogenetic affiliation to *Dechloromonas* spp. (**Table S1; Fig. S3**). This MAG is named here "*Candidatus* Dechloromonas occultata" sp. nov.; etymology: occultata; (L. fem. adj. 'hidden'). The remaining 10% of proteins mapped to

Deltaproteobacteria; of those, 70% mapped to a nearly complete MAG (*Desulfuromonadales* bacterium GT-UBC; NCBI accession RHLS01000000) with 80% ANI to *Geobacter sulfurreducens*. This MAG is named here "*Candidatus* Geobacter occultata".

Cytochromes containing multiple *c*-type hemes are key for electron transport during microbial metal transformations, and therefore might also be expected to play a role in Mn(III) reduction. Numerous mono-, di-, and multi (>3)-heme cytochromes (MHCs) were expressed by "*Ca*. D. occultata" in Mn(III)-reducing cultures. Nine out of 15 MHCs encoded by the "*Ca.* D. occultata" MAG were expressed, including two decahemes similar to MtoA in Fe(II)-oxidizing *Betaproteobacteria* (**Tables 1, S2, S3; Figs. 2A, S4).** Several highly expressed MHCs were encoded on a previously unreported 19-gene cluster with 10 cytochrome-*c* proteins, hereafter *occA-S* (**Table 1; Figs. 2B, S5, S6**). OccP was an undecaheme, which are known to be involved in metal reduction (6, 21). "*Ca*. Dechloromonas occultata" may reduce Mn(III) using the novel extracellular undecaheme OccP as the terminal Mn(III) reductase, although the function of the putative Occ complex has yet to be experimentally verified.

We investigated the taxonomic distribution of genes encoding *occP*, *mtoA*, and key denitrification complexes expressed by "*Ca*. D. occultata". We discovered *occP* homologs (40-60% identity) in diverse *Betaproteobacteria* from diverse freshwaters and deep subsurface groundwaters, as well as several *Gammaproteobacteria* and one alphaproteobacterium (**Fig. 2D; Table S3).** Most *occP*-containing bacteria also possessed *mtoA* and denitrification (**Fig. 2D**) genes. For more discussion of denitrification, see **Supplemental Text** and **Figs. S7** and **S8**.

While the specific role of CH_4 in Mn(III) reduction remains unknown (see **Supplemental Text**), CH_4 significantly stimulated expression of many cytochrome *c* proteins, including OccABGJK, MtoD-2, and cytochrome-*c*4 and -*c*5 proteins associated with anaerobic respiration

(p < 0.05; **Table 1; Fig. 2C**). Methane also led to increased expression of several "*Ca.* D. occultata" proteins involved in outer membrane structure and composition, including an extracellular DUF4214 protein located next to an S-layer protein similar to those involved in manganese binding and deposition (24), a serine protease possibly involved in Fe(III) particle attachment (3), an extracellular PEP-CTERM sorting protein for protein export (9), and a Tol-Pal system for outer membrane integrity (**Table 1)**. A type IV pilin protein (87% identity to *Geobacter pickeringii* (14)) was significantly more highly expressed with CH_4 vs. N_2 in the "*Ca.* G. occultata" proteome (p=0.02; **Table 1**). The possible involvement of *Geobacter* e-pilins in Mn(III) reduction remains an open question. For additional discussion of other features of "*Ca.* D. occultata" and "*Ca.* G. occultata" genomes and proteomes, see **Supplemental Text**, **Table S4**, Figs. **S9**, **S10**, and **S11**.

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References

1 Anantharaman K, Brown CT, Hug LA, Sharon I, Castelle CJ, Probst AJ *et al* (2016). Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. *Nat Commun* **7:** 13219.

2 Beal EJ, House CH, Orphan VJ (2009). Manganese- and iron-dependent marine methane oxidation. *Science* **325:** 184-187.

3 Burns JL, Ginn BR, Bates DJ, Dublin SN, Taylor JV, Apkarian RP *et al* (2009). Outer membrane-associated serine protease involved in adhesion of Shewanella oneidensis to Fe (III) oxides. *Environmental science & technology* **44:** 68-73.

4 Crowe SA, Katsev S, Leslie K, Sturm A, Magen C, Nomosatryo S *et al* (2011). The methane cycle in ferruginous Lake Matano. *Geobiology* **9:** 61-78.

5 Cummings DE, Caccavo F, Spring S, Rosenzweig RF (1999). Ferribacterium limneticum, gen. nov., sp. nov., an Fe(III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. *Archives of Microbiology* **171:** 183-188.

6 Edwards MJ, Hall A, Shi L, Fredrickson JK, Zachara JM, Butt JN *et al* (2012). The crystal structure of the extracellular 11-heme cytochrome UndA reveals a conserved 10-heme motif and defined binding site for soluble iron chelates. *Structure* **20:** 1275-1284.

7 Emerson D, Field EK, Chertkov O, Davenport KW, Goodwin L, Munk C *et al* (2013). Comparative genomics of freshwater Fe-oxidizing bacteria: implications for physiology, ecology, and systematics. *Front Microbiol* **4:** 254.

8 Finneran KT, Johnsen CV, Lovley DR (2003). Rhodoferax ferrireducens sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). *International Journal of Systematic and Evolutionary Microbiology* **53:** 669-673.

9 Haft DH, Paulsen IT, Ward N, Selengut JD (2006). Exopolysaccharide-associated protein sorting in environmental organisms: the PEP-CTERM/EpsH system. Application of a novel phylogenetic profiling heuristic. *BMC Biol* **4:** 29.

10 Hansel C (2017). Small but mighty: how minor components drive major biogeochemical cycles. *Environmental microbiology reports* **9:** 8-10.

11 Hartshorne RS, Reardon CL, Ross D, Nuester J, Clarke TA, Gates AJ *et al* (2009). Characterization of an electron conduit between bacteria and the extracellular environment. *Proc Natl Acad Sci U S A* **106:** 22169-22174.

12 He S, Barco RA, Emerson D, Roden EE (2017). Comparative Genomic Analysis of Neutrophilic Iron (II) Oxidizer Genomes for Candidate Genes in Extracellular Electron Transfer. *Frontiers in Microbiology* **8:** 1584.

13 Hernsdorf AW, Amano Y, Miyakawa K, Ise K, Suzuki Y, Anantharaman K *et al* (2017). Potential for microbial H2 and metal transformations associated with novel bacteria and archaea in deep terrestrial subsurface sediments. *The Isme Journal* **11:** 1915.

14 Holmes DE, Dang Y, Walker DJ, Lovley DR (2016). The electrically conductive pili of Geobacter species are a recently evolved feature for extracellular electron transfer. *Microbial Genomics* **2**.

15 Jones C, Crowe SA, Sturm A, Leslie KL, MacLean LCW, Katsev S *et al* (2011). Biogeochemistry of manganese in ferruginous Lake Matano, Indonesia. *Biogeosciences* **8:** 2977-2991.

16 Kato S, Ohkuma M, Powell DH, Krepski ST, Oshima K, Hattori M *et al* (2015). Comparative genomic insights into ecophysiology of neutrophilic, microaerophilic iron oxidizing bacteria. *Frontiers in Microbiology* **6:** 1265.

17 Kostka JE, Luther III GW, Nealson KH (1995). Chemical and biological reduction of Mn (III)-pyrophosphate complexes: potential importance of dissolved Mn (III) as an environmental oxidant. *Geochimica et Cosmochimica Acta* **59:** 885-894.

18 Oldham VE, Siebecker MG, Jones MR, Mucci A, Tebo BM, Luther GW (2019). The Speciation and Mobility of Mn and Fe in Estuarine Sediments. *Aquatic Geochemistry*.

19 Riedinger N, Formolo MJ, Lyons TW, Henkel S, Beck A, Kasten S (2014). An inorganic geochemical argument for coupled anaerobic oxidation of methane and iron reduction in marine sediments. *Geobiology* **12:** 172-181.

20 Shi L, Dong H, Reguera G, Beyenal H, Lu A, Liu J *et al* (2016). Extracellular electron transfer mechanisms between microorganisms and minerals. *Nature Reviews Microbiology* **14:** 651.

21 Smith JA, Lovley DR, Tremblay PL (2013). Outer cell surface components essential for Fe(III) oxide reduction by Geobacter metallireducens. *Appl Environ Microbiol* **79:** 901-907.

22 Szeinbaum N, Burns JL, DiChristina TJ (2014). Electron transport and protein secretion pathways involved in Mn (III) reduction by *Shewanella oneidensis*. *Environmental Microbiology Reports* **6:** 490-500.

23 Szeinbaum N, Lin H, Brandes JA, Taillefert M, Glass JB, DiChristina TJ (2017). Microbial manganese(III) reduction fuelled by anaerobic acetate oxidation. *Environ Microbiol* **19:** 3475-3486.

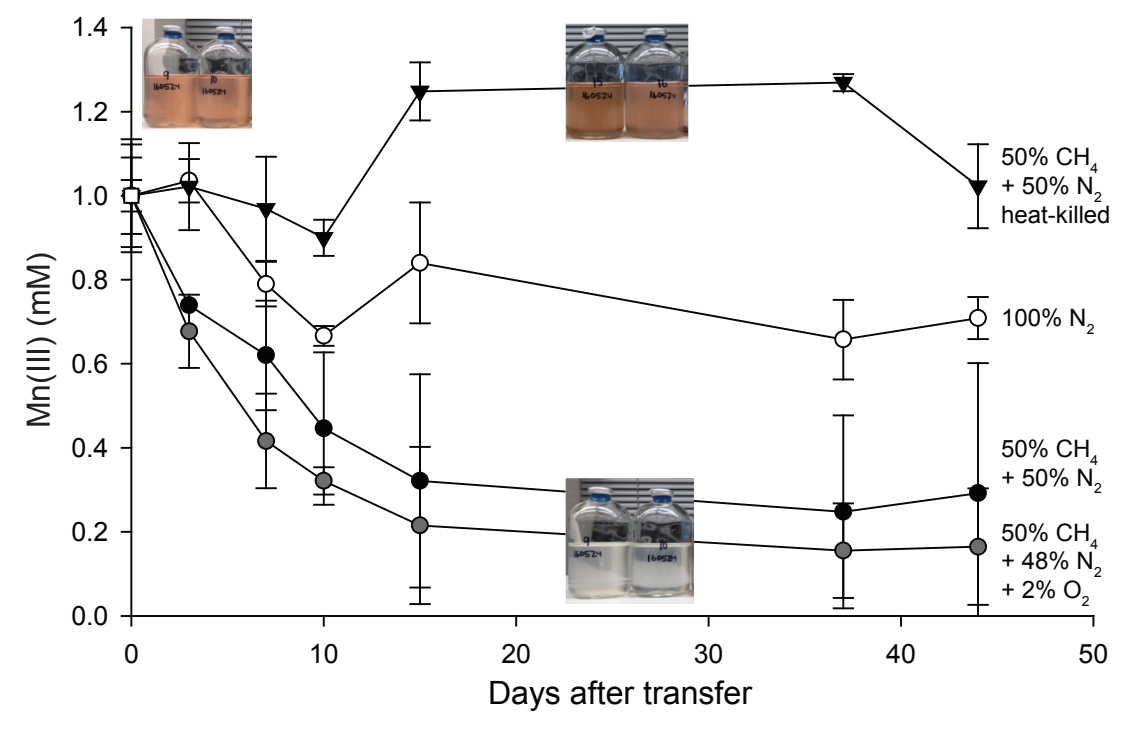
24 Wang X, Schröder HC, Schloßmacher U, Müller WE (2009). Organized bacterial assemblies in manganese nodules: evidence for a role of S-layers in metal deposition. *Geo-Marine Letters* **29:** 85-91.

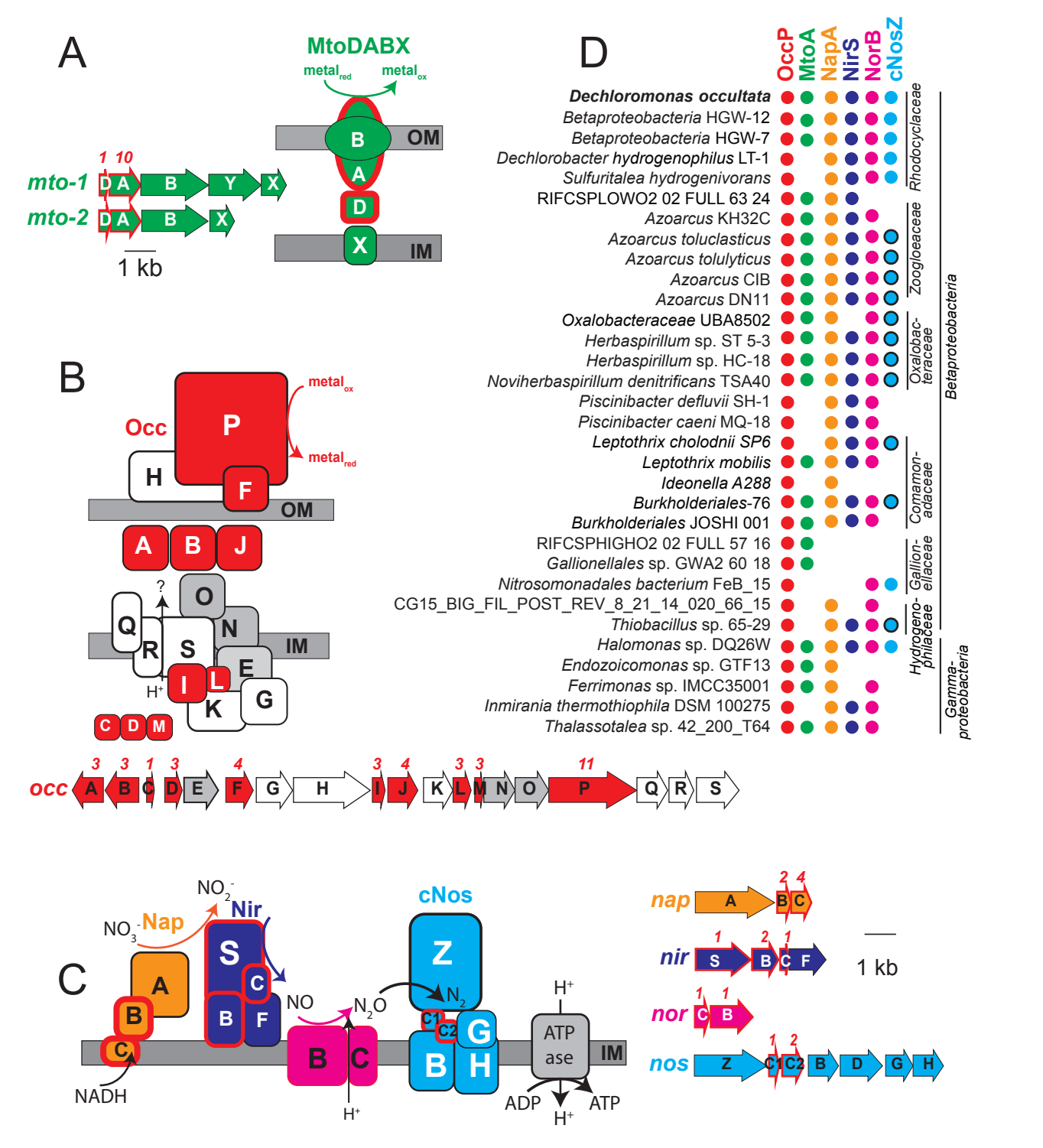
Table and Figure Captions

Table 1. Expression levels for "*Ca*. D. occultata" proteins in the presence of CH_4 and N_2. Peptide counts are normalized to total "*Ca*. D. occultata" proteins x 10,000. Blank cells indicate proteins with <2 normalized peptide counts. Gray boxes indicate membrane proteins with that may be underrepresented by mass spectrometry-based metaproteomic analyses, which inherently favor soluble over insoluble membrane-bound or hydrophobic proteins. SP: signal peptide (Y:present/N:absent); TMH: numbers of transmembrane helices; # CxxCH: number of heme-binding motifs; P-sort: predicted cellular location. Bold proteins indicate proteins that were significantly more expressed with CH_4 than N_2 ($CH_4/N_2>1$; $p<0.05$). MCP: methyl-accepting chemotaxis protein; PPIase: Peptidyl-proline isomerase; P: periplasm, C: cytoplasm; OM: outer membrane; IM: inner membrane, E: extracellular; U: unknown. MtoX and MtoY were predicted to be an inner membrane cytochrome-b protein and a methyl-accepting chemotaxis protein, respectively.

Figure 1. Consumption of Mn(III) in Lake Matano enrichments in the presence and absence of methane. Sediment-free cultures (transfer 4) from 335 days after the initial enrichment were incubated for 45 days with 1 mM Mn(III) pyrophosphate as the sole electron acceptor. Initial bottle headspace contained 50% CH_4 + 50% N_2 (black circles), 50% CH_4+48% N_2+2% O_2 (gray circles), 100% N_2 (white circles), and 50% CH_4+50% N_2 heat killed controls (black triangles). Error bars are standard deviations from duplicate experiments. Color change from red to clear indicates Mn(III) reduction.

Figure 2. Gene arrangement, predicted protein locations, and taxonomic distribution of major expressed respiratory complexes in "*Ca*. D. occultata". A: MtoDAB(Y)X porin-cytochrome c electron conduit; **B:** OccA-S; **C:** denitrification complexes (Nap, Nir, Nor and cNos); **D:** Occurrence of key marker genes in *Betaproteobacteria* and *Gammaproteobacteria* with >95% complete genomes that encode OccP. Red fill around genes and proteins indicate cytochrome-*c* proteins. Black outlines around blue circles in D indicate type I nitrous oxide reductase to distinguish from blue dots (type II/cytochrome-nitrous oxide reductase). Gray-shaded genes on the *occ* gene cluster indicate 6-NHL repeat proteins. Protein locations shown are based on P-sort predictions. Numbers above genes indicate number of CxxCH motifs predicted to bind cytochrome *c*. IM: inner membrane; OM: outer membrane. For more details, see **Table 1** and **Table S3.**


Mn(III) (mM)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
Days after transfer
50% CH4 + 50% N2 heat-killed
100% N2
50% CH4 + 50% N2
50% CH4 + 48% N2 + 2% O2


A
MtoDABX
metal_red
metal_ox
B
OM
A
D
X
IM
1 10
mto-1
D A B Y X
mto-2
D A B X
1 kb
B
Occ
P
H
F
OM
metal_ox
metal_red
A B J
?
O
Q
N
R
S
IM
E
I
L
K
G
H+
C D M
occ
3 3 1 3 4
A B C D E F G H
3 4 3 3 11
I J K L M N O P Q R S
D
OccP MtoA NapA NirS NorB cNosZ
Dechloromonas occultata
Betaproteobacteria HGW-12
Betaproteobacteria HGW-7
Dechlorobacter hydrogenophilus LT-1
Sulfuritalea hydrogenivorans
RIFCSPLOWO2 02 FULL 63 24
Azoarcus KH32C
Azoarcus toluclasticus
Azoarcus tolulyticus
Azoarcus CIB
Azoarcus DN11
Oxalobacteraceae UBA8502
Herbaspirillum sp. ST 5-3
Herbaspirillum sp. HC-18
Noviherbaspirillum denitrificans TSA40
Piscinibacter defluvii SH-1
Piscinibacter caeni MQ-18
Leptothrix cholodnii SP6
Leptothrix mobilis
Ideonella A288
Burkholderiales-76
Burkholderiales JOSHI 001
RIFCSPHIGHO2 02 FULL 57 16
Gallionellales sp. GWA2 60 18
Nitrosomonadales bacterium FeB_15
CG15_BIG_FIL_POST_REV_8_21_14_020_66_15
Thiobacillus sp. 65-29
Halomonas sp. DQ26W
Endozoicomonas sp. GTF13
Ferrimonas sp. IMCC35001
Inmirania thermothiophila DSM 100275
Thalassotalea sp. 42_200_T64
Rhodocyclaceae
Zoogloeaceae
Oxalobac-teraceae
Comamon-adaceae
Gallion-ellaceae
Hydrogeno-philaceae
Betaproteobacteria
Gamma-proteobacteria
C
NO3- Nap
NO2- Nir
A
B
C
S
B F
C
NADH
NO
N2O
cNos
Z
C1 C2
G
B H
N2
B C
H+
ATP ase
ADP
ATP
IM
nap A 2 4 B C
nir 1 S 2 B 1 C F
nor 1 1 C B
nos Z 1 2 C1 C2 B D G H
1 kb

Enzyme	Function	SP	TMH	CxxCH	P-sort	NCBI ID	Normalized peptide counts				CH4/N2		P value
							CH_4	SD	N_2	SD	ave	SD	
***Ca.* Dechloromonas occultata**													
Mto-1	MtoX-1 (cyt-b)	N	5	0	IM	RIX49676							
	MtoY-1 (MCP)	N	2	1	IM	RIX49677	2.7	0.5	3.6	0.2	0.8	0.2	0.2
	MtoB-1 (porin)	Y	0	0	OM	RIX49678	10	2	15	2	0.6	0.1	0.004
	MtoA-1	Y	1	10	P	RIX49874	5	1	2.5	0.1	1.9	0.4	0.1
	MtoD-1	N	0	1	P	RIX49875							
Mto-2	MtoX-2 (cyt-b)	N	4	0	IM	RIX48942							
	MtoB-2 (porin)	Y	0	0	OM	RIX48943	8	1	16	0.2	0.5	0.1	0.04
	MtoA-2	Y	1	10	P	RIX48944	7.3	0.8	4	2	2.1	1.3	0.2
	MtoD-2	**Y**	**1**	**1**	**U**	**RIX48945**	**2.6**	**0.3**	**0.7**	**0.3**	**4.0**	**1.4**	**0.003**
Occ	**OccA**	**Y**	**1**	**3**	**P**	**RIX49688**	**4**	**0.5**	**0.7**	**0.6**	**7.8**	**5.7**	**0.01**
	OccB	**Y**	**0**	**3**	**U**	**RIX49689**	**41**	**4**	**19**	**2**	**2.2**	**0.0**	**0.03**
	OccC	N	0	1	U	RIX49877							
	OccD	N	0	3	U	RIX49878							
	OccE (6-NHL)	N	1	0	U	RIX49690	22	2.1	20.5	0.2	1.1	0.1	0.2
	OccF	Y	2	4	E	RIX49691	13	0.7	10.1	0.1	1.3	0.1	0.06
	OccG (PPIase)	**N**	**0**	**0**	**U**	**RIX49692**	**14**	**1**	**3.3**	**0.5**	**4.2**	**0.3**	**0.01**
	OccH	N	0	0	OM/E	RIX49693	6.0	0.2	7.7	0.6	0.8	0.1	0.10
	OccI	N	1	3	U	RIX49694	7	2.5	2.3	0.0	2.9	1.1	0.1
	OccJ	**Y**	**0**	**4**	**U**	**RIX49879**	**44**	**0.2**	**19**	**3**	**2.4**	**0.4**	**0.03**
	OccK	**N**	**0**	**0**	**C**	**RIX49880**	**39**	**6**	**13**	**1**	**3.0**	**0.2**	**0.04**
	OccL	N	1	3	U	RIX49695							
	OccM	N	0	3	U	RIX49881							
	OccN (6 NHL)	N	2	0	U	RIX49696	5.7	0.3	6	1	0.9	0.1	0.2
	OccO (6 NHL)	N	0	0	U	RIX49882	1.2	0.8	4.2	0.4	0.3	0.2	0.03
	OccP	N	0	11	E	RIX49697	14	2	12	3	1.2	0.5	0.4
	OccQ	Y	4	0	IM	RIX49698							
	OccR	N	8	0	IM	RIX49883							
	OccS	N	12	0	IM	RIX49699							
Cyt c	**Cyt c5**	**N**	**1**	**1**	**U**	**RIX47670**	**27**	**2**	**9**	**3**	**3.2**	**0.8**	**0.01**
	Cyt c5	Y	1	2	P	RIX40984	19	2	6	1	**3.3**	**1.0**	0.06
	Cyt c'/C_2	Y	1	1	P	RIX44710	17	5	3.6	0.8	**4.8**	**2.3**	0.09
	Cyt c'/C_2	Y	1	1	P	RIX49630	7	1	1.2	0.9	**8.2**	**6.6**	0.07
	Cyt c551/c552	Y	0	1	P	RIX49087	13	3	2.8	0.0	**4.8**	**1.1**	0.06
	Cyt c4	Y	0	2	P	RIX48804	16	0.8	9.8	0.8	**1.6**	**0.2**	0.06
	Cyt c4	Y	0	2	P	RIX44782	4	2	1.7	0.7	**2.6**	**0.1**	0.08
	Cyt c4	**Y**	**0**	**2**	**P**	**RIX45018**	**7**	**0.6**	**2.2**	**0.2**	**3.0**	**0.0**	**0.02**
Nap	NapA	Y	0	0	P	RIX41011	76	2	67	3	1.1	0.1	0.1
	NapB	**Y**	**1**	**2**	**P**	**RIX41010**	**15**	**1**	**5**	**2**	**3.2**	**0.9**	**0.02**
	NapC	N	1	4	IM	RIX41009	12	3	13	1	1.0	0.2	0.1
Nir	NirS	Y	0	1	P	RIX44719	58	2	44	4	1.3	0.2	0.1
	NirB	Y	1	2	P	RIX44720	14	3	10	2	1.5	0.6	0.2
	NirC	N	0	1	P	RIX44788							
	NirF	Y	1	0	P or C	RIX44721	2	1	7	1	0.3	0.1	0.02
Nor	NorC	N	1	1	IM	RIX45182	3.5	0.7	3.2	0.7	1.1	0.0	0.1
	NorB	N	12	1	IM	RIX45183							
cNos	cNosZ	Y	0	0	P	RIX42539	77	17	66	8	1.2	0.3	0.2
	cNosC1	Y	1	1	P	RIX42538	16	2	4	2	5	3	0.08
	cNosC2	**Y**	**1**	**2**	**P**	**RIX42537**	**10**	**0.1**	**3.9**	**0.3**	**2.6**	**0.1**	**0.02**
	cNosB	N	6	0	IM	RIX42536							
	cNosD	N	0	0	P	RIX42535							
	cNosG	N	1	0	C	RIX42534							
	cNosH	N	4	0	IM	RIX42533							
Qcr	QcrA	N	9	0	CM	RIX41976							
	QcrB	N	9	0	CM	RIX41977							
	QcrC	N	1	0	CM	RIX41978							
Proteases	**Serine protease**	**N**	**0**	**0**	**P**	**RIX49468**	**27**	**2**	**1.0**	**0.3**	**29**	**10**	**0.02**
	Carboxyl-terminal protease (S41)	**N**	**1**	**0**	**CM**	**RIX48818**	**18.5**	**0.8**	**8.0**	**0.9**	**2.3**	**0.1**	**0.0002**
Membrane/Extracellular	**DUF4214 protein**	**N**	**0**	**0**	**OM/E**	**RIX44180**	**146**	**25**	**43**	**0.6**	**3.4**	**0.5**	**0.05**
	S-layer protein	N	0	0	U	RIX44181	8	0.5	10	0.6	0.8	0.1	0.14
	PEP-CTERM sorting	**Y**	**1**	**0**	**E**	**RIX45463**	**68**	**6**	**33**	**10**	**2.1**	**0.5**	**0.03**
	Tol-Pal system protein TolB	**Y**	**0**	**0**	**P**	**RIX44015**	**20**	**2**	**12**	**1**	**1.67**	**0.05**	**0.03**
	Peptidoglycan-associated lipoprotein (Pal)	**N**	**0**	**0**	**OM**	**RIX44016**	**27.3**	**0.2**	**10**	**3**	**3**	**1**	**0.04**
	Tol-Pal system protein YbgF	Y	0	0	U	RIX44017	10.8	0.4	4	2	4	2	0.06
	Pilus assembly protein	**N**	**0**	**0**	**U**	**RIX46961**	**54**	**5**	**30**	**5**	**1.8**	**0.1**	**0.001**
Other	**PQQ-dependent dehydrogenase**	**Y**	**0**	**0**	**P**	**RIX45050**	**37**	**4**	**17**	**1**	**2.2**	**0.1**	**0.03**
	Phasin family granule-associated protein	**N**	**0**	**0**	**U**	**RIX40682**	**49**	**2**	**22**	**1**	**2.2**	**0.2**	**0.03**
	Phasin family granule-associated protein	**Y**	**0**	**0**	**U**	**RIX40683**	**34**	**4**	**16**	**1**	**2.1**	**0.0**	**0.03**
	High potential iron-sulfur protein	**Y**	**0**	**0**	**U**	**RIX49681**	**10.79**	**0.01**	**6.5**	**0.4**	**1.7**	**0.1**	**0.02**
	Electron transfer flavoprotein (FixA)	**N**	**0**	**0**	**C**	**RIX43544**	**16**	**3**	**10**	**2**	**1.7**	**0.0**	**0.04**
***Ca.* Geobacter occultata**													
E-pilus	**Type IV pilin PilA**	**N**	**1**	**0**	**E**	**RNC67631**	**93**	**3**	**18**	**3**	**5**	**1**	**0.02**