Supplemental tables and figure Qiu et al., 2013

Table S1 Bacterial strains and plasmids

Strain	Description	Source or reference
Escherichia coli WM3064	*thrB1004 pro thi rpsL hsdS lacZDM15 RP4-1360 (araBAD)567 dapA1341::[erm pir(wt)]*	W. Metcalf (1)
E. coli TOP10	F2 *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80*lacZ*DM15 Δ *lacX74 deoR recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL* (Sm^r) *endA1 nupG*	Invitrogen
E. coli EC100D+	*F^- mcrA Δ(mrr-hsdRMS-mcrBC) φ80dlacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ- rpsL (Str^R) nupG pir^+(DHFR)*	Epicentre Technologies
Shewanella putrefaciens W3-18-1	Dissimilatory metal-reducing strain (Pacific Ocean marine sediments)	Murray *et al*., 2001 (2)
W3-18-1ΔpstI	In-frame deletion mutant of pstI gene (Sputw3181_4075)	This study
W3-18-1ΔpstIM	In-frame deletion mutant of pstI gene and pstM (Sputw3181_4074)	This study
W3-18-1Δnapα	In-frame deletion mutant of *nap-α* (*napDABC*) genes (Sputw 3181_2102-2107)	This study
W3-18-1Δnapβ	In-frame deletion mutant of *nap-β* (*napDAGHB*) genes (Sputw3181_0792-0796)	This study
W3-18-1ΔnapαβΔ	Double mutant with in-frame deletion mutant of *nap-α* and *nap-β* genes	This study
W3-18-1ΔcymA	In-frame deletion mutant of *cymA* (Sputw3181_ 3916)	This study
S. oneidensis MR-1	Dissimilatory metal-reducing strain (Lake Oneida, NY)	Myers & Nealson, 1988 (3)
Plasmid		
pDS3.0	Suicide vector derived from pCDV224; *Amp^r, Gm^r, sacB*	Wan *et al*., 2004 (4)
pBBR1MCS-2	Broad-host-range vector, Km^r, *lacZ*	Kovach *et al*., 1995 (5)
pBBR1MCS-5	Broad-host-range vector, Gm^r, *lacZ*	Kovach *et al*., 1995 (5)

Table S2 Primers used in this study

Primer	Nucleotide sequence
PstIko_5O	AGAGCTCTGGGCTGAATCGGTAACTTC
PstIko_5I	CTATGTCGGATGCTGGCCTAATGTCGGTTACTTGGCTTGG
PstIko_3I	TAGGCCAGCATCCGACATAGTCTCTGCCCAGAATCTTGCT
PstIko_3O	AGAGCTCGTATCCGGGCAAGCTAAACA
Pstmko_5O	AGAGCTCGACTTAAAATCGCCAGGTCT
Pstmko_5I	CTATGTCGGATGCTGGCCTA ACCAAACAGTGAGCGACTAA
Pstko_Lf	TCAAGGCATTACGTGAGCTG
Pstko_Lr	AGAGCTCGCTTTCCACTTTCAGGATCT
Napαko_5O	AGAGCTCGGGCATTAAAAAGCGCCTTA
Napαko_5I	GTTCACTGAACGACATAAACCACTCATGCGTCCTCCAAGA
Napαko_3I	GTTTATGTCGTTCAGTGAACTATCTCCAGCCAAAGGCCAA
Napαko_3O	AGAGCTCATAATCGAGGGGAAAATCCG
Napαko_Lf	GCTCAAGATGCACACCGTCA
Napαko_Lr	AAGATGCATTGGCATCAGGC
Napβko_5O	AGAGCTCGCCGTGACGGTAAAGTTGC
Napβko_5I	GACTGGCTTAGGTCGTCTCTCGGGTGACCTTTAGGTGGT
Napβko_3I	AGAGACGACCTAAGCCAGTCCCAATAGACGATACTCCCCTG
Napβko_3O	AGAGCTCGCAGCCAAATCCACCGTAT
Napβko_Lf	ATCCAAGGTTACCGTTTCGA
Napβko_Lr	CAAATTAGTGCGAGATCCCA
CymAko_5O	AGCGTTTCATCGTTACAGCA
CymAko_5I	GATAACATTAAGTTACACC TGCACGCCAGTTCATTACTCT
CymAko_3I	GGTGTAACTTAATGTTATCCTTTTGAGGGCTTTTTGCTG

CymAko_3O	TGTACTCGATTGTTTCGGCG
CymA_F	ACTCGAGCTAATTTTGGCAATTTTGGAGA
CymA_R	GGTCGACTTATCCTTTTGGATATGGGTGA
crpko_5O	AGAGCTCATAAATCAGCCAGCGTTTGC
crpko_5I	TGCATCGAGTTGATTGTCGCTCAGAGCCATGTTGATGTTCC
crpko_3I	GCGACAATCAACTCGATGCAGTCTTCAGCTTGATTTAAGCCTG
crpko_3O	AGAGCTCTTTACTGTTTGCTCCGTTTCA
crp_F	AGAATTCGAGGAACATCAACATGGCTCTG
crp_R	ACTGCAGAATTTATGCTAGGCCACTTTAATGA
RT-PCR primers	
16S-F	GTTGGAAACGACTGCTAATACC
16S-R	GGTCCTTCTTCTGTAGGTAACG
RT_napAα-F	TCTTTGGGTGGGTTTGTGGC
RT_napAα-R	CGGGGAGATGGTGGGCTATT
RT_napAβ-F	GGATTAGGCTTAACGGCTACGGC
RT_napAβ-R	TGTGGGTAAAAACATGGCGTG

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Table S3 Comparison of *c*-type cytochromes between W3-18-1 and MR-1

No.	Cytochrome	Orthologous gene		Presence in the *Shewanella putrefaciens* CN-32 strains
		W3-18-1	MR-1	
1	Diheme *c4*	Sputw3181_0043	SO_4666	Sputcn32_3908
2	CoxIIc Diheme *c*	Sputw3181_0133	SO_4606	Sputcn32_3782
3	ScyA Monoheme *c5*	Sputw3181_0189	SO_0264	Sputcn32_3726
4	SHP Monoheme *c*	Sputw3181_0209	SO_4484	Sputcn32_0359
5	Diheme *c*	Sputw3181_0210	SO_4485	Sputcn32_0360
6	Tetraheme *c*	Sputw3181_0555	Absent	Sputcn32_3388
7	Hypothetical Seven-heme *c*	Sputw3181_0577	SO_0479	Sputcn32_3364
8	PetC-Monoheme *c1*	Sputw3181_0667	SO_0610	Sputcn32_3274
9	NapB Diheme *c*	Sputw3181_0792	SO_0845	Sputcn32_3151
10	Monoheme *c*	Sputw3181_1274	SO_3420	Sputcn32_2738
11	OmcA-like Decaheme *c*	Sputw3181_1501	Frameshift	Sputcn32_2507
12	Split Tetraheme Flavocytochrome	Sputw3181_1578	SO_3056	Sputcn32_2430
13	STC Small Tetraheme *c*	Sputw3181_1675	SO_2727	Sputcn32_2333
14	BCCP Diheme *c*	Sputw3181_1721	SO_2178	Sputcn32_2287
15	FixO/CcoO Monoheme *c*	Sputw3181_2047	SO_2363	Sputcn32_1958
16	FixP/CcoP Diheme *c*	Sputw3181_2049	SO_2361	Sputcn32_1956
17	NapC Tetraheme	Sputw3181_2103	Absent	Sputcn32_1905
18	NapB Diheme *c*	Sputw3181_2104	Absent	Sputcn32_1906
19	Tetraheme Cytochrome *c3*	Sputw3181_2184	Absent	Sputcn32_1825
20	OmcA-like 11 heme *c* (UndA1 or OmcE)	Sputw3181_2622	SO_1779 (OmcA1)	Sputcn32_1479
21	MtrC/OmcB Decaheme *c*	Sputw3181_2623	SO_1778	Sputcn32_1478
22	MtrA Decaheme *c*	Sputw3181_2624	SO_1777	Sputcn32_1477
23	OmcA-like Decaheme *c*	Sputw3181_2721	SO_1659	Sputcn32_1380

24	Decaheme Cytochrome *c*	Sputw3181_2977	Absent	Sputcn32_1187
25	FccA Tetraheme Flavocytochrome	Sputw3181_3267	SO_0970	Sputcn32_0905
26	Split-Soret Diheme	Sputw3181_3294	SO_0939	Sputcn32_0879
27	NrfA Pentaheme *c*	Sputw3181_3486	SO_3980	Sputcn32_0685
28	SoxA-like Diheme *c*	Sputw3181_3546	SO_4047	Sputcn32_0624
29	Diheme *c4*	Sputw3181_3547	SO_4048	Sputcn32_0623
30	NrfA-like Pentaheme *c*	Sputw3181_3743	Absent	Sputcn32_3604
31	NrfB Pentaheme *c*	Sputw3181_3895	Deleted	Sputcn32_0313
32	CymA Tetraheme *c*	Sputw3181_3916	SO_4591	Sputcn32_0286
33	Monoheme *c*, Acceptor for Molybdopterin	Absent	SO_0714	Absent
34	Monoheme *c*	Absent	SO_0716	Absent
35	Monoheme *c4*	Absent	SO_0717	Absent
36	TorC pentaheme *c*	Absent	SO_1233	Absent
37	Split Tetraheme flavocytochrome	Absent	SO_1413	Absent
38	IfcA Tetraheme flavocytochrome	Disrupted by transposon	SO_1421	Sputcn32_0810 (Sputw3181_3363 was disrupted by insertion of sputw3181_3364)
39	MtrAD-like Decaheme *c*	Absent	SO_1427	Absent
40	MtrF Decaheme *c*	Absent	SO_1780	Absent
41	MtrD Decaheme *c*	Absent	SO_1782	Absent
42	Hypothetical Diheme *c*	Absent	SO_2930	Absent
43	Hypothetical Diheme *c*	Absent	SO_2931	Absent
44	Split Tetraheme Flavocytochrome	Absent	SO_3300	Absent
45	Split Tetraheme Flavocytochrome	Absent	SO_3623	Sputcn32_3535

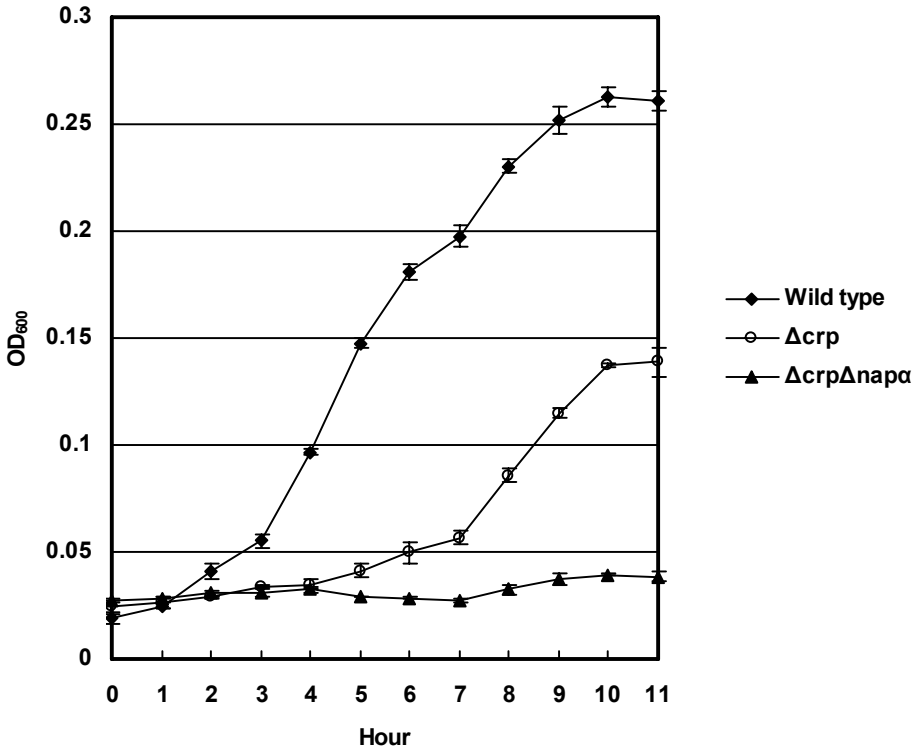
46	Monoheme *c*	Absent	SO_4142	Absent
47	Octaheme Tetrathionate Reductase (Otr)	Absent	SO_4144	Absent
48	MtrAD-like Decaheme	Absent	SO_4360	Absent
49	Monoheme *c*	Absent	SO_4570	Absent
50	Triheme *c*	Absent	SO_4572	Absent

Table S4 Comparison of respiration chain components and related genes between MR-1 and W3-18-1

Gene or gene cluster	Functions	Locus		Presence in the CN-32 strain
		W3-18-1	MR-1	
fdh-N operon	Nitrate reductase-linked formate dehydrogenase, proton pump	Absent	SO_0101 - SO_0113	Absent
fdh-O operons	Formate dehydrogenase-O, proton pump, encoded by two contiguous *fdoGHI* gene cassettes	Sputw3181_3870 - 3873	SO_4508 - SO_4511	Sputcn32_0338 - 0335
		Sputw3181_3874 - 3877	SO_4512 - SO_4515	Sputcn32_0334 - 0331
fdh-H	Hydrogenase-linked formate dehydrogenase	Sputw3181_0894	SO_0988	Sputcn32_3051
hydAB operon	Fe-only hydrogenase, hydrogen production	Absent	SO_3920 - SO_3926	Absent
hyaAB operon	Ni-Fe hydrogenase, hydrogen utilization	Separated into two gene clusters: Sputw3181_1919 -1924; sputw3181_2173-2178	SO_2089 - SO_2099	Separated into two gene clusters: Sputcn32_2093 – 2088; Sputcn32_1836 - 1831
ndh-I operon	NADH dehydrogenase I (NuoA-NuoN), proton pump	Absent	SO_1009 - SO_1021	Absent
ndh-II	NADH dehydrogenase II	Sputw3181_3093	SO_3517	Sputcn32_1702
		Sputw3181_2863	Deleted in MR-1	Sputcn32_1241
nqrABCDEF-1	Sodium ion translocating NADH dehydrogenase I	Sputw3181_3324 - 3319	SO_0902 - SO_0907	Sputcn32_0849 - 0854
nqrABCDEF-2	Sodium ion translocating NADH dehydrogenase II	Sputw3181_3236 - 3230	SO_1103 - SO_1109	Sputcn32_0946 - 0940
rnfABCDGE	Electron transfer complex RnfABCDGE	Sputw3181_2159 - 2164	SO_2508 - SO_2513	Sputcn32_1850 - 1845
otr operon	Octaheme tetrathionate reductase Otr	Absent	SO_4142 - SO_4144	Absent
ttr operon	Tetrathionate reductase TtrACB	Sputw3181_3510 - 3512	Absent	Sputcn32_0664- 0662
psrABC	Polysulfide reductase PsrABC	Sputw3181_3557-3559	SO_4060 - SO_4062	Sputcn32_0613- 0611
sorAB operon	Sulfite hydrogenase SorAB and monoheme cytochromes, sulfite oxidation	Absent	SO_0714, 0715(*sorA*), 0716 (*sorB*), 0717	Absent
nap-alpha	Periplasmic nitrate reductase NapDABC	Sputw3181_2103-2107	Absent	Sputcn32_1901- 1905
nap-beta	Periplasmic nitrate reductase NapDAGHB	Sputw3181_0792-0796	SO_0845 - SO_0849	Sputcn32_3151- 3147
norZ-norR	Nitric oxide reductase NorZ and regulator NorR	Sputw3181_0872 (*norR*) -0873 (*norZ*)	Absent	Sputcn32_3073 - 3072
cyoABCDE	Cytochrome *bo* terminal oxidase, proton pump	Sputw3181_0091-0096	Absent	Sputcn32_3862 - 3857
cioAB	Cyanide insensitive cytochrome *bd* terminal oxidase	Sputw3181_3246-3247	Absent	Sputcn32_0930 - 0929
torECADSTR	Trimethylamine N-oxide reductase	Absent	SO_1228 - SO_1234	Absent

dms	Dimethyl sulfoxide reductase I, Dms-1	Absent	SO_1427 - SO_1432	Absent
	Dimethyl sulfoxide reductase II, Dms-2	Absent	SO_4357 - SO_4362	
mtrABC	Dissimilatory metal reductase	Sputw3181_2623-2625	SO_1776 -SO_1778	Sputcn32_1478 - 1476
mtrDEF	Secondary metal reductase	Absent	SO_1780 - SO_1782	Absent

Figure S1



Supplemental Figure S1 Anaerobic growth (OD_{600}) of wild type strain of *S. putrefaciens* W3-18-1, *Δcrp* mutant, and double mutant of *Δcrp* and *Δnap-alpha* on nitrate (2mM) in modified M1 minimum media supplemented with 50 mM of sodium lactate as the electron donor and carbon source.

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