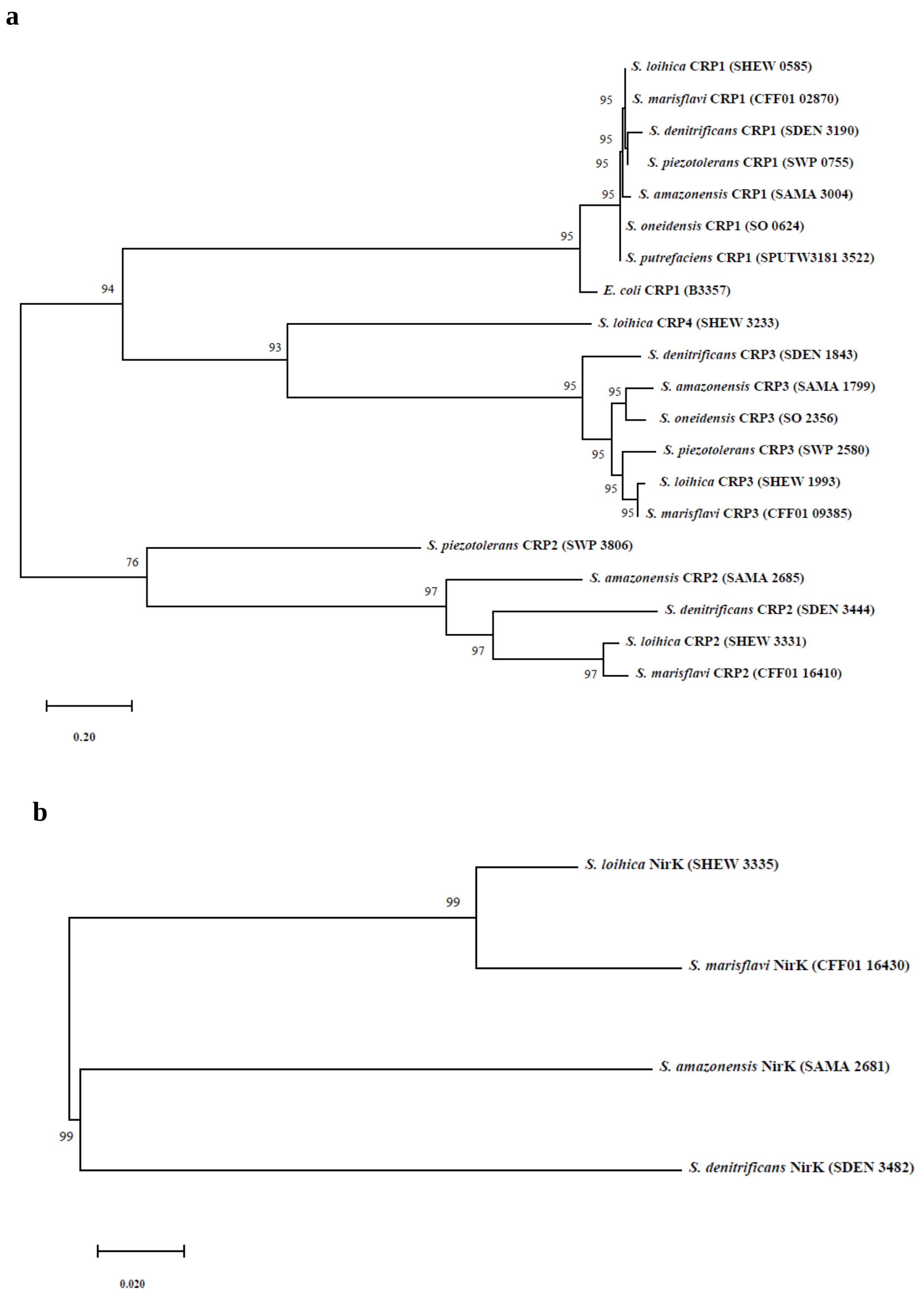
Supplemental Tables and Figures

Supplemental Table S1 The presence of *napAB*, *nrfAH/nirBD*, *nirK*, *norBC* and *nosZ* orthologs in sequenced *Shewanella* genomes

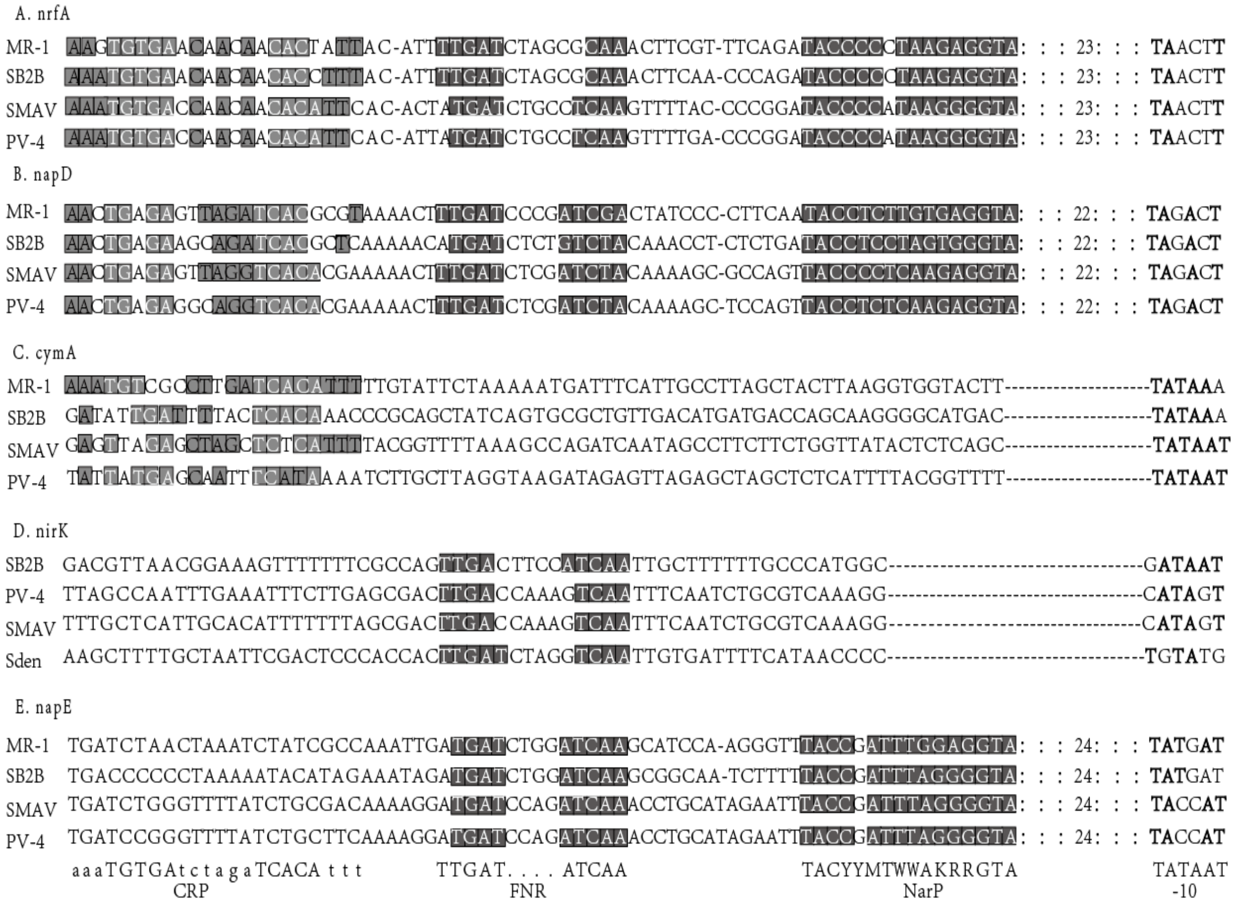
Strains	**Genes**				
	napAB	***nrfAH/nirBD***	***nirK***	***norBC***	***nosZ***
S. oneidensis	SO_0845 SO_0848	SO_3980			
S. denitrificans	Sden_1497 Sden_1498	Sden_3715 Sden_3716	Sden_3482	Sden_1980 Sden_1981	Sden_2219
S. frigidimarina	Sfri_0552 Sfri_0553 Sfri_2552 Sfri_2553	Sfri_0622 Sfri_3036 Sfri_1509 Sfri_1510		Sfri_3253	
S. amazonensis	Sama_1622 Sama_1623 Sama_2983 Sama_2986	Sama_0648	Sama_2681	Sama_0728	
S. baltica OS155	Sbal175_0854 Sbal175_0855 Sbal175_2442 Sbal175_2443	Sbal175_3632			
S. loihica	Shew_1770 Shew_1771 Shew_3206 Shew_3209	Shew_0505 Shew_0844	Shew_3335	Shew_0657	Shew_3400
S. putrefaciens CN-32	Sputcn32_1903 Sputcn32_1904 Sputcn32_3148 Sputcn32_3151	Sputcn32_0685 Sputcn32_3604		Sputcn32_3072	
S. putrefaciens 200	Sput200_2087 Sput200_2088 Sput200_3259 Sput200_3262	Sput200_0617 Sput200_2562 Sput200_3617		Sput200_3181	
S. sp. *ANA-3*	Shewana3_1900 Shewana3_1901 Shewana3_3430 Shewana3_3433	Shewana3_0658 Shewana3_2363		Shewana3_0846	
S. sp. *W3-18-1*	Sputw3181_0792 Sputw3181_0795 Sputw3181_2104 Sputw3181_2105	Sputw3181_3486 Sputw3181_3743		Sputw3181_0873	

Table S2. CRP/FNR homologues in in *Shewanella loihica* PV-4, *Escherichia coli* K-12, *Shewanella oneidensis* MR-1, *Shewanella* sp. W3-18-1, *Shewanella denitrificans*, *Shewanella amazonensis*, *Marinobacter psychrophilus*, *Marinobacter* sp. BSs20148, *Pseudomonas* sp. CCOS 191 and *Pseudomonas aeruginosa* LESB58

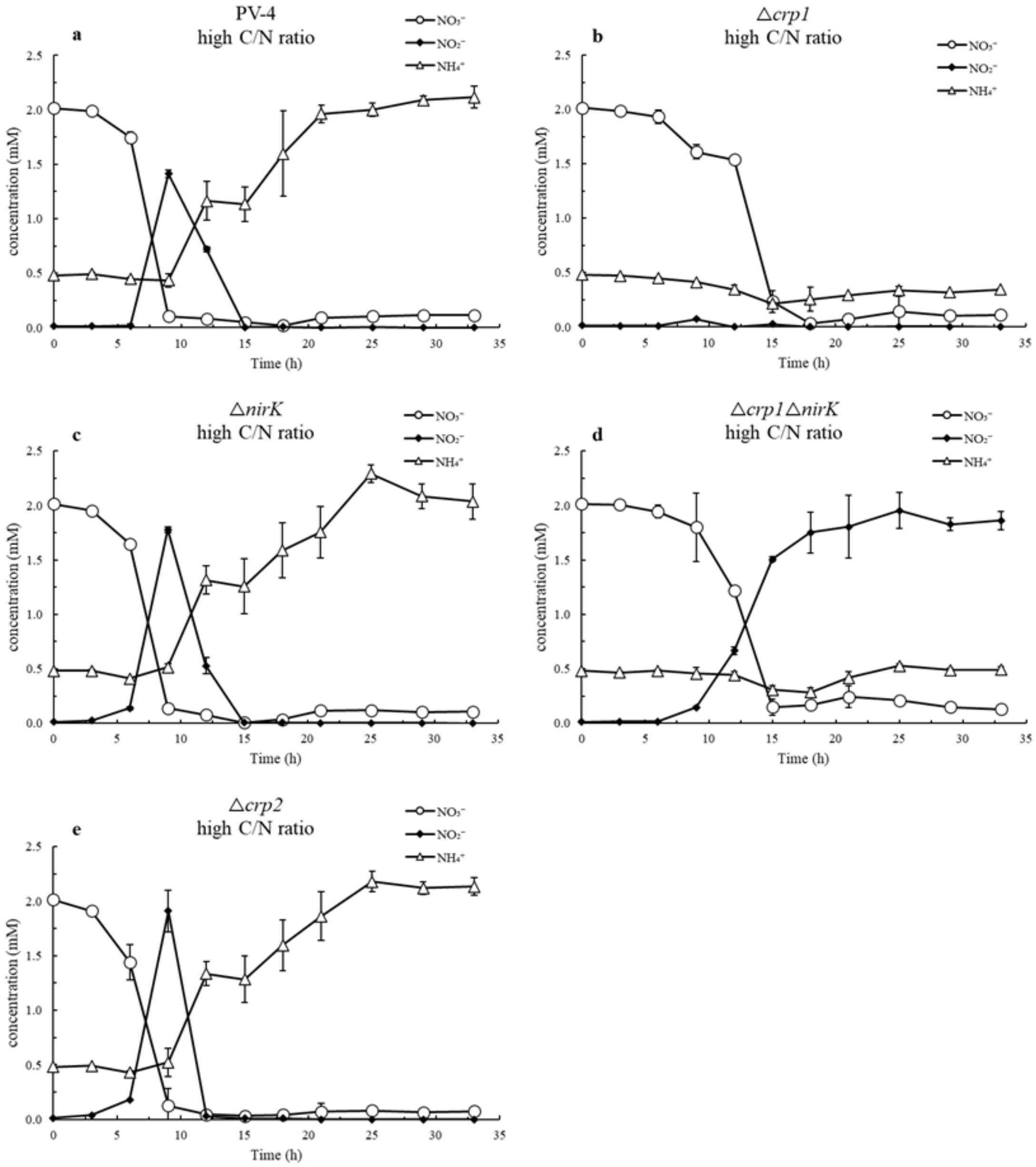
	crp1	***crp2***	***crp3***	***crp4***	***nirK/nirS***
Shewanella loihica PV-4	*Shew_0585*	*Shew_3331*	*Shew_1993*	*Shew_3233*	*Shew_3335*
Shewanella oneidensis MR-1	*SO_0624*	-	*SO_2356*	-	-
Shewanella* sp. *W3-18-1	*Sputw3181_3522*	-	*Sputw3181_2054*	-	-
Escherichia coli K-12	*b3357*	-	*b1334*	-	-
Pseudomonas aeruginosa LESB58	*PLES_06311*	*PLES_05241*	*PLES_37841*	-	*PLES_05161*
Pseudomonas* sp. *CCOS 191	*CCOS191_4899*	*CCOS191_4267*	*CCOS191_3685*	-	*CCOS191_0291*
Marinobacter* sp. *BSs20148	*MRBBS_3547*	*MRBBS_3132*	*MRBBS_0830*	-	*MRBBS_1013*
Marinobacter psychrophilus	*ABA45_16645*	*ABA45_14855*	*ABA45_04340*	-	*ABA45_05245*
Shewanella denitrificans	*Sden_3190*	*Sden_3444*	*Sden_1843*	-	*Sden_3482*
Shewanella amazonensis	*Sama_3004*	*Sama_2685*	*Sama_1799*	-	*Sama_2681*



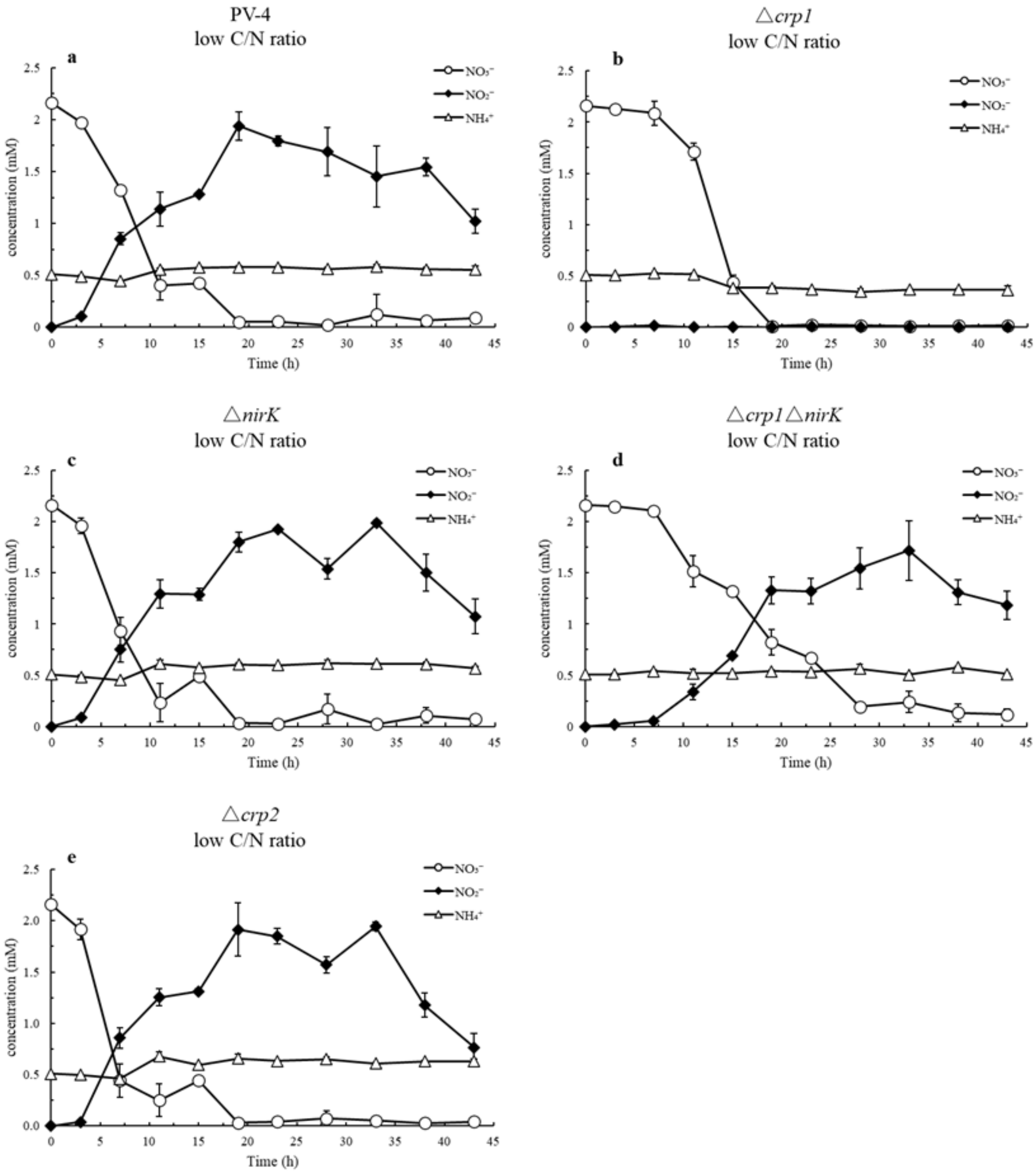
Supplemental Figure S1 (a), Distance tree of CRP paralogues in different *Shewanella* species based on protein sequences similarity. *E. coli* CRP1 used as reference. (b), Distance tree of NirK in different *Shewanella* species based on protein sequences similarity. Bootstrap values (expressed as percentages of 1000 replications) ≥50% are shown at branch points.



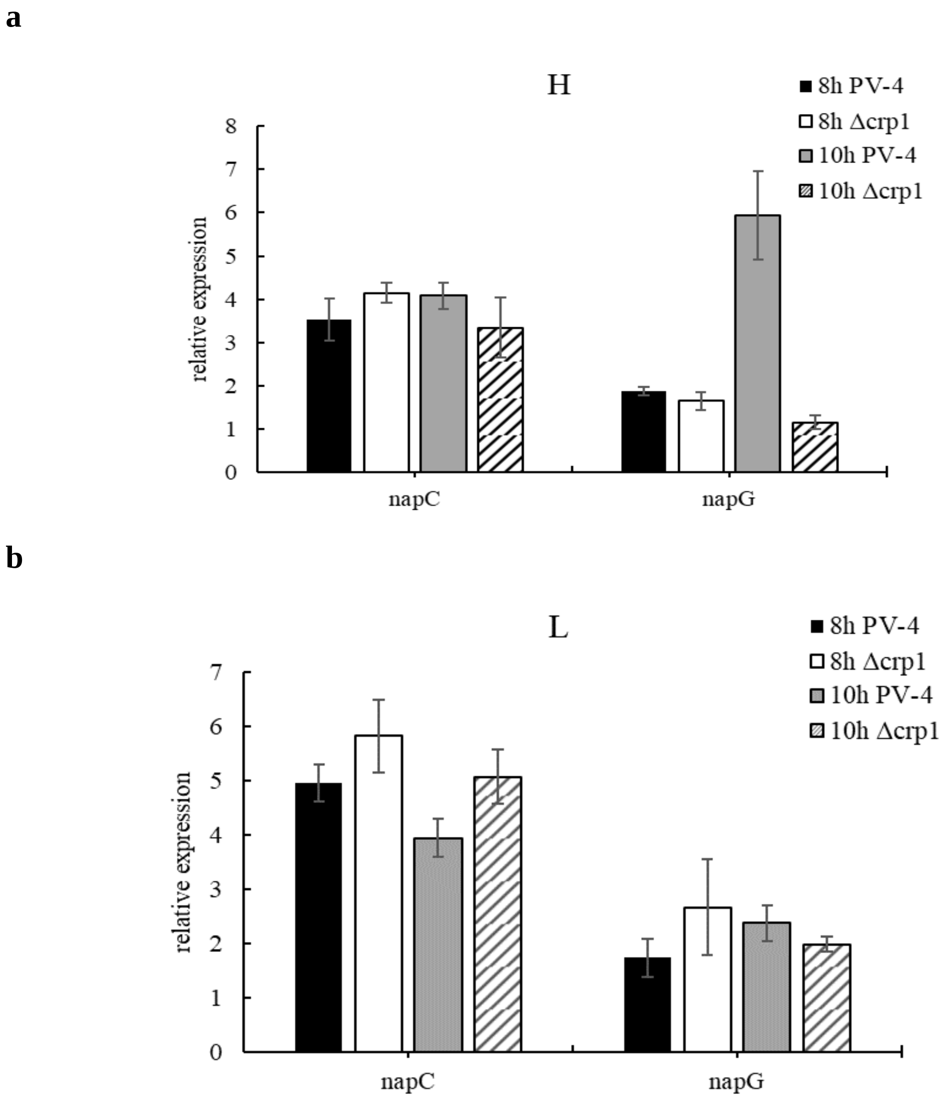
Supplemental Figure S2. Multiple sequence alignment of the 5'-upstream regions of *nap-alpha*, *nir*K, nap beta, *cym*A and *nrf*A genes. Sequence motifs recognized by CRP1, FNR and NarP are indicated below the alignment, with the most conserved sequences indicated in capital letters and others in small letters. Y, C or T; M, A or C; K, G or T; W, A or T; and R, G or A. The motifs in the upstream regions are shaded; in white for the capital letters and in black for the small letters. MR1, *Shewanella oneidensis*; SB2B, *Shewanella amazonensis*; SMAV, *Shewanella marisflavi*; PV4, *Shewanella loihica*. (1)



Supplemental Figure S3. The nitrate reduction of wild type strain of *S. loihica* PV-4 (a), in-frame deletion mutants Δ*crp1* (b), Δ*nirK* (c), *Δcrp1ΔnirK* double mutant (d) and in-frame deletion mutants Δ*crp2* (e) under high carbon/nitrogen ratios. The concentration of NO_3^-, NO_2^-, NH_4^+ in the medium was measured under high carbon/nitrogen ratio (C/N=12), respectively. Lactate (10 mM) and nitrate (2 mM) were added to phosphate-buffered basal salt medium supplemented with 0.5 mM of ammonium chloride as nitrogen source. Results are the mean of three replicates and error bars indicates standard deviation.

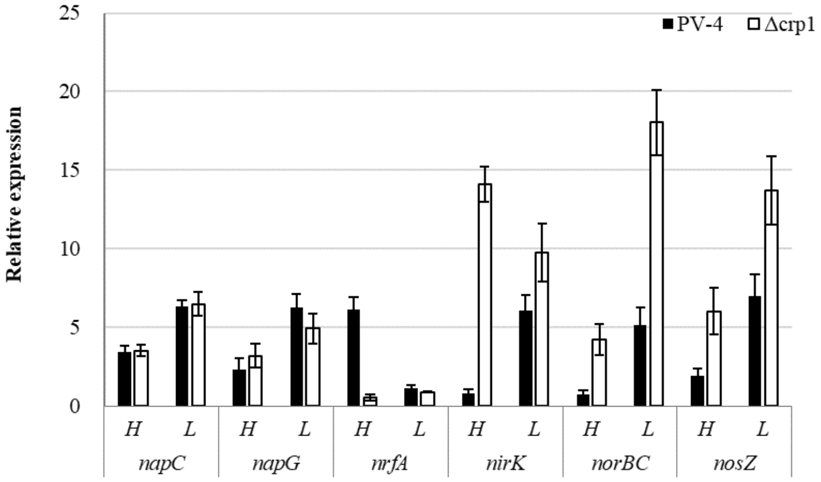


Supplemental Figure S4. The nitrate reduction of wild type strain of *S. loihica* PV-4 (a), in-frame deletion mutants Δ*crp1* (b), Δ*nirK* (c), *ΔcrpIΔnirK* double mutant (d) and in-frame deletion mutants Δ*crp2* (e) under low carbon/nitrogen ratios. The concentration of NO_3^-, NO_2^-, NH_4^+ in the medium was measured under low carbon/nitrogen ratio (C/N=2), respectively. Lactate (1.66 mM) and nitrate (2 mM) were added to phosphate-buffered basal salt medium supplemented with 0.5 mM of ammonium chloride as nitrogen source. Results are the mean of three replicates and error bars indicates standard deviation.

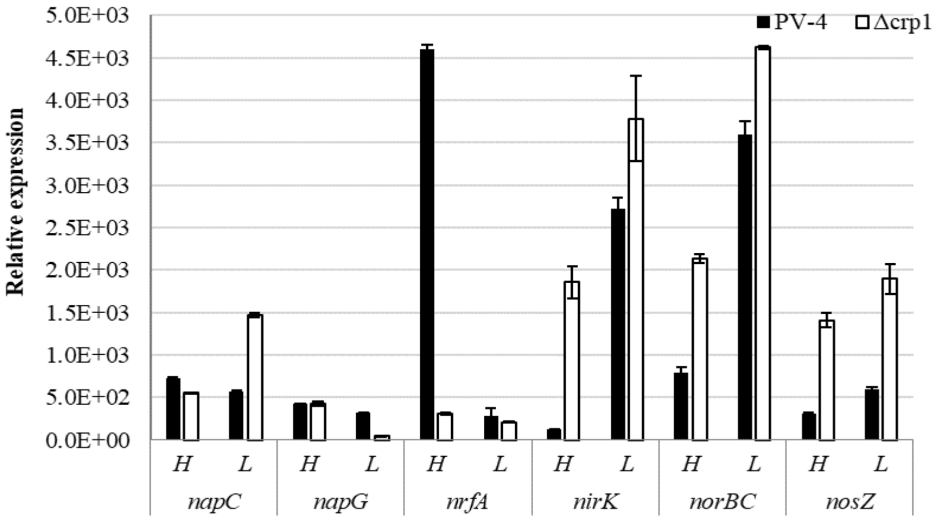


Supplemental Figure S5. The quantitative PCR analyses on transcriptional profile of nitrate reduction genes *napC* (CRP-independent *nap-alpha* gene cluster) and *napG* (CRP1-dependent *nap-beta* gene cluster) in the wild type strain *S. loihica* PV-4 and in-frame deletion mutant Δ*crp1* under high carbon/nitrogen ratios (a) and low carbon/nitrogen ratios (b) at 8 and 10 hours after anaerobic cultivation. H represents C/N=55, L represents C/N=4. Gene expression was normalized against the 16S rRNA gene using the $2^{-\Delta CT}$ calculation: $\Delta C_T = C_{T\text{gene of interest}} - C_{T\text{16S rRNA}}$ (C_T, threshold cycle). Results are the mean of three replicates and error bars indicate standard deviation.

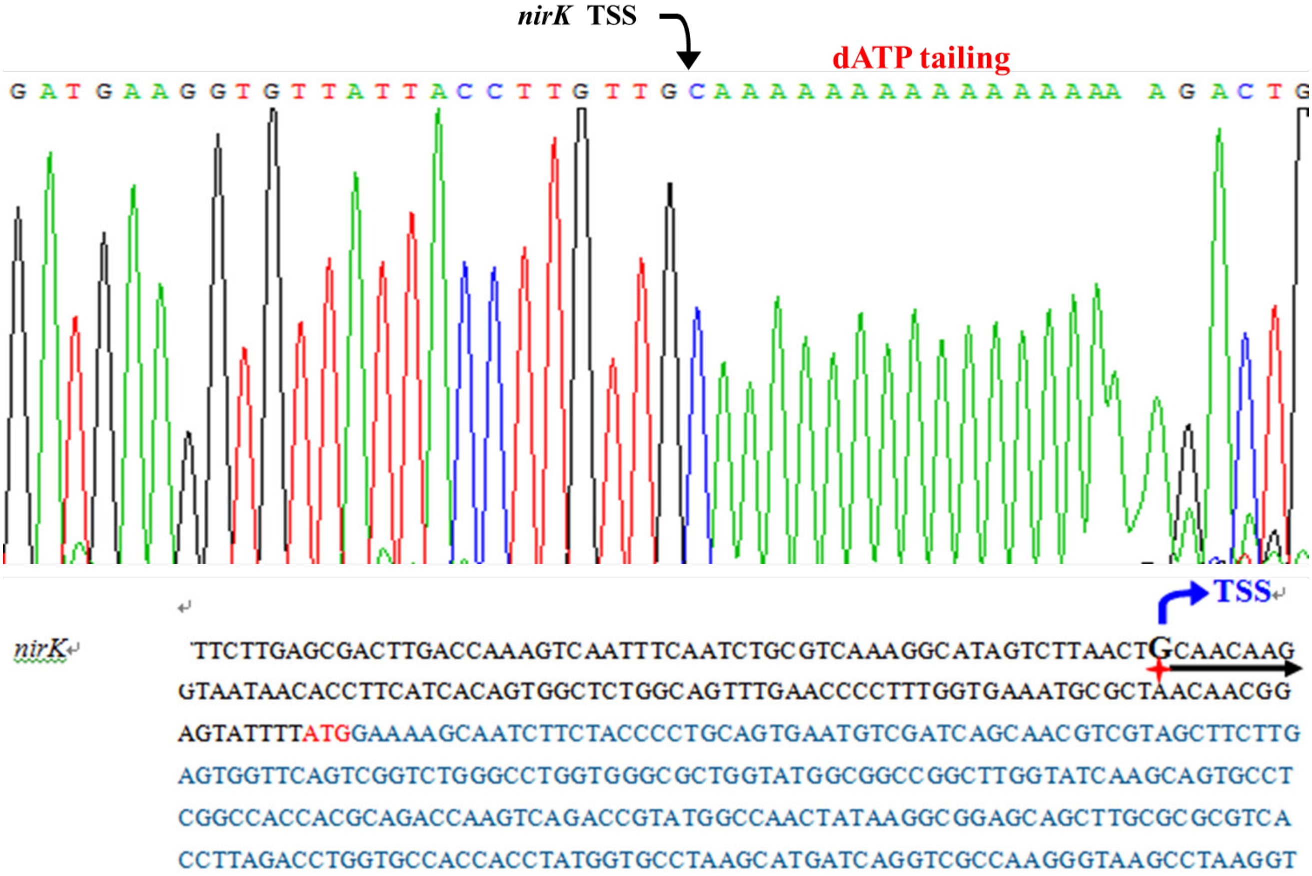
a



b



Supplemental Figure S6. The quantitative PCR analyses on transcriptional profile of nitrate reduction genes in the wild type strain *S. loihica* PV-4 and in-frame deletion mutant Δ*crp1* under different carbon/nitrogen ratios at 14 hours after anaerobic cultivation. The tests were repeated twice (a and b). Gene expression was normalized against the 16S rRNA gene using the $2^{-\Delta CT}$ calculation: $\Delta C_T = C_{T\text{gene of interest}} - C_{T\text{16S rRNA}}$ (C_T, threshold cycle). H represents high carbon/nitrogen ratio, L represents low carbon/nitrogen ratio. Results are the mean of three replicates and error bars indicate standard deviation.

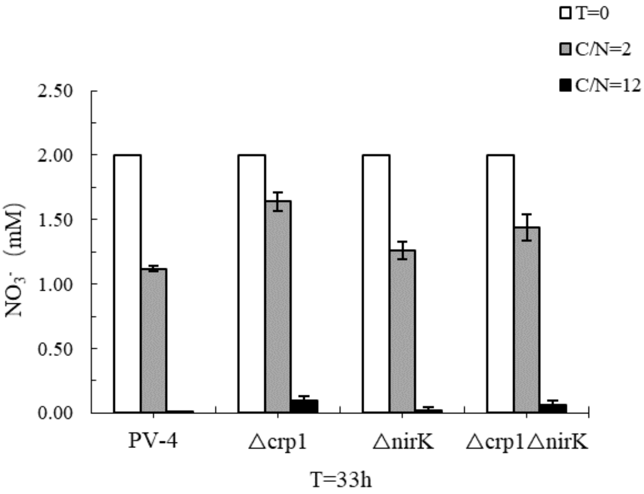


nirK

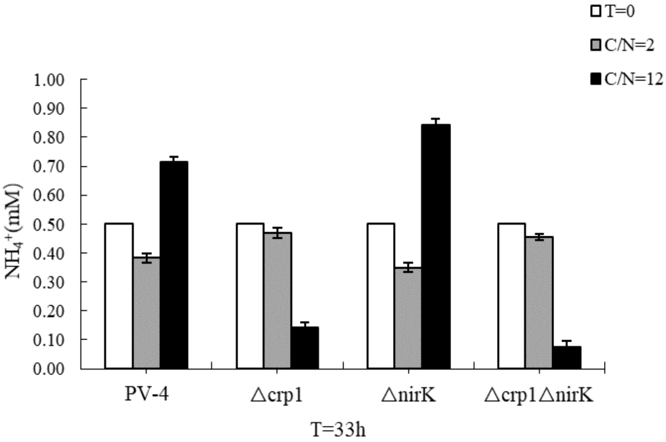
TTCTTGAGCGACTTGACCAAAGTCAATTTCAATCTGCGTCAAAGGCATAGTCTTAACTGCAACAAG
GTAATAACACCTTCATCACAGTGGCTCTGGCAGTTTGAACCCCTTTGGTGAAATGCGCTAACAACGG
AGTATTTTATGGAAAAGCAATCTTCTACCCCTGCAGTGAATGTCGATCAGCAACGTCGTAGCTTCTTG
AGTGGTTCAGTCGGTCTGGGCCTGGTGGGCGCTGGTATGGCGGCCGGCTTGGTATCAAGCAGTGCCT
CGGCCACCACGCAGACCAAGTCAGACCGTATGGCCAACTATAAGGCGGAGCAGCTTGCGCGCGTCA
CCTTAGACCTGGTGCCACCACCTATGGTGCCTAAGCATGATCAGGTCGCCAAGGGTAAGCCTAAGGT

Supplemental Figure S7. Primer extension analysis of transcriptional start site (TSS) of the *nirK*.

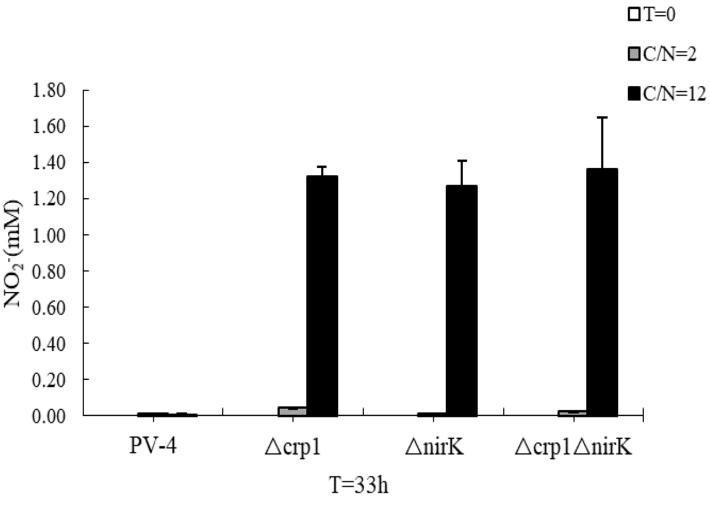
a


☐ T=0
▨ C/N=2
■ C/N=12
NO_3^- (mM)
2.50
2.00
1.50
1.00
0.50
0.00
PV-4
△crp1
△nirK
△crp1△nirK
T=33h

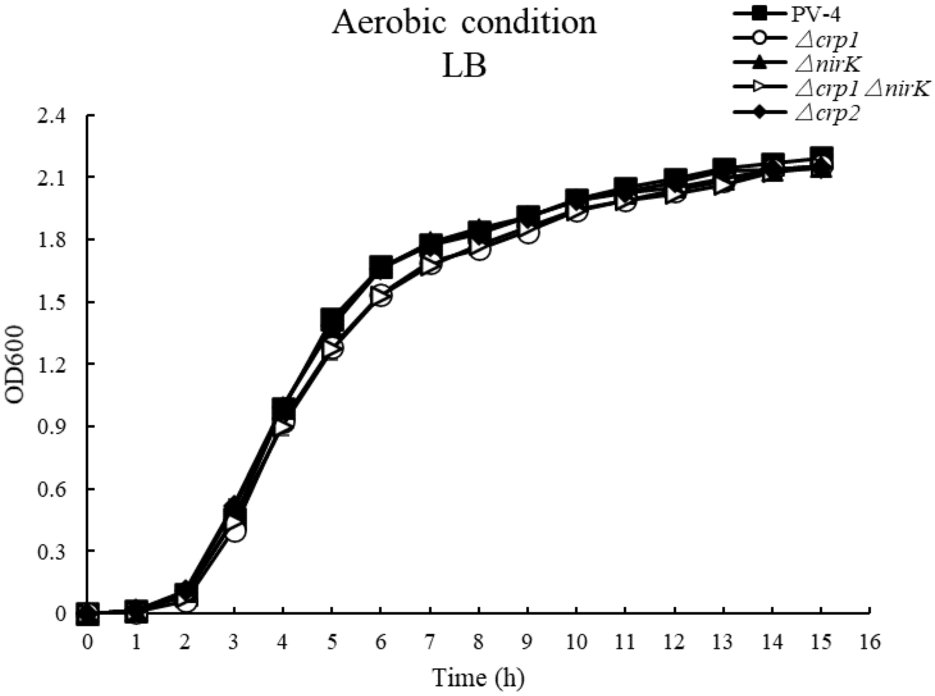
b


☐ T=0
▨ C/N=2
■ C/N=12
NH_4^+(mM)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
PV-4
△crp1
△nirK
△crp1△nirK
T=33h

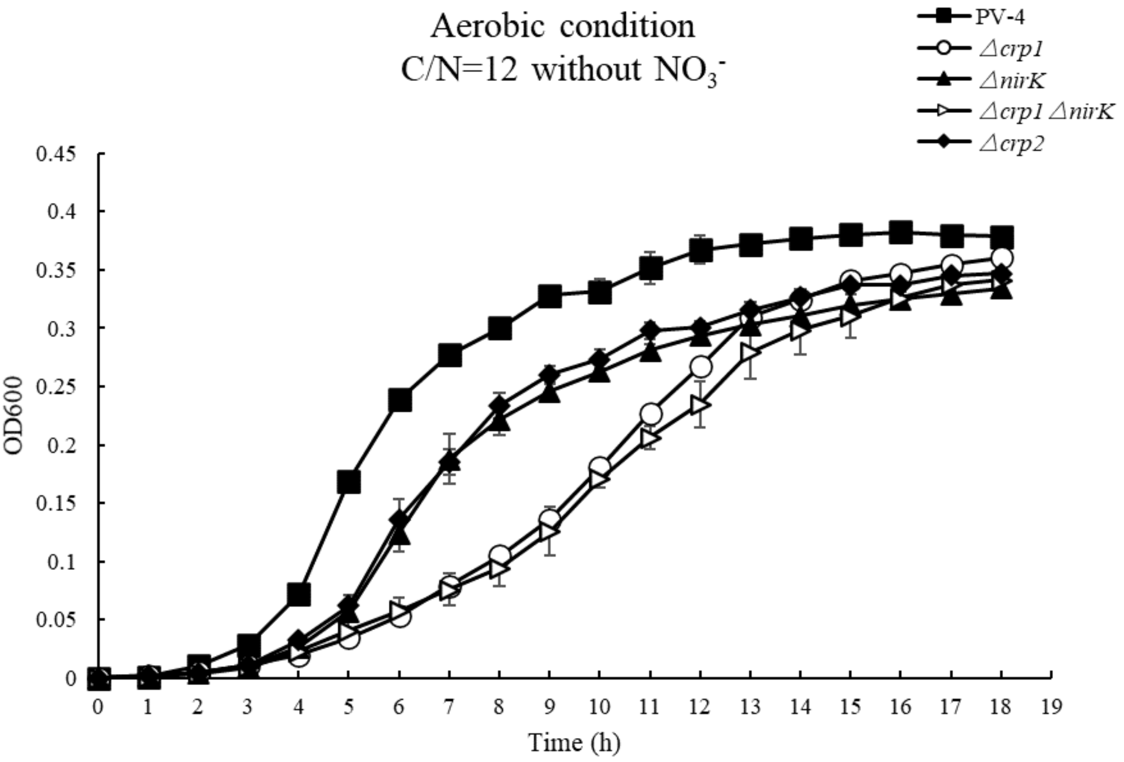
c


☐ T=0
▨ C/N=2
■ C/N=12
NO_2^-(mM)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
PV-4
△crp1
△nirK
△crp1△nirK
T=33h

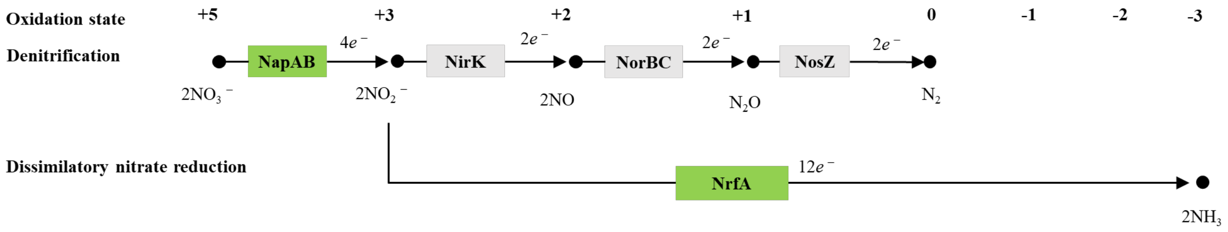
Supplemental Figure S8. The nitrate (a), ammonium (b) and nitrite (c) concentrations of the *S. loihica* PV-4, in-frame deletion mutants Δ*crp1*, Δ*nirK*, and *Δcrp1ΔnirK* double mutant grown under different carbon/nitrogen ratios.



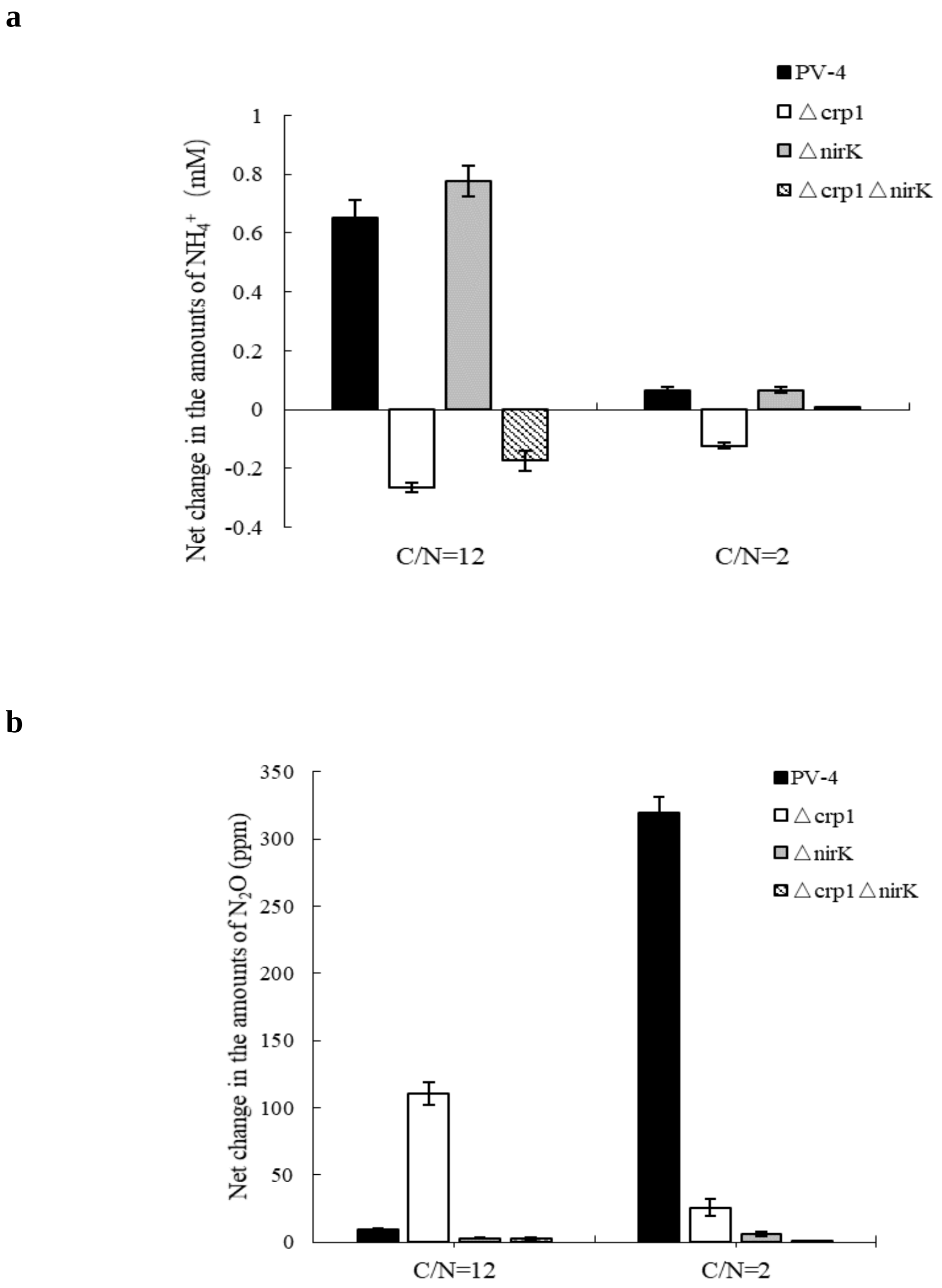
Supplemental Figure S9. Bacterial growth (optical density at 600 nm [OD_{600}]) of the *S. loihica* PV-4, in-frame deletion mutants Δ*crp1*, Δ*crp2*, Δ*nirK*, and Δ*crp1*Δ*nirK* double mutant in the rich medium (LB broth) under aerobic condition.



Supplemental Figure S10. Bacterial growth (optical density at 600 nm [OD_{600}]) of the *S. loihica* PV-4, in-frame deletion mutants Δ*crp1*, Δ*crp2*, Δ*nirK*, and Δ*crp1*Δ*nirK* double mutant under aerobic condition and high carbon/nitrogen ratio. Lactate (10 mM) was added to phosphate-buffered basal salt medium supplemented with 0.5 mM of ammonium chloride as nitrogen source without nitrate.



Supplemental Figure S11. Schematic diagram of electron transport and chemical valence change of the nitrate reduction (DNRA and denitrification) in *S. loihica* PV-4.



Supplemental Figure S12. Comparison of the degree change magnitude of ammonium (a) and nitric oxide (b) levels in the cultures of the wild type strain of *Shewanella loihica* PV-4, in-frame deletion mutants Δ*crp1*, Δ*nirK*, and Δ*crp1*Δ*nirK* double mutant grown under different carbon/nitrogen ratios.

References:

1. **Stewart V, Bledsoe PJ, Chen L-L, Cai A**. 2009. Catabolite repression control of napF (periplasmic nitrate reductase) operon expression in *Escherichia coli* K-12. J Bacteriol **191**:996-1005.