

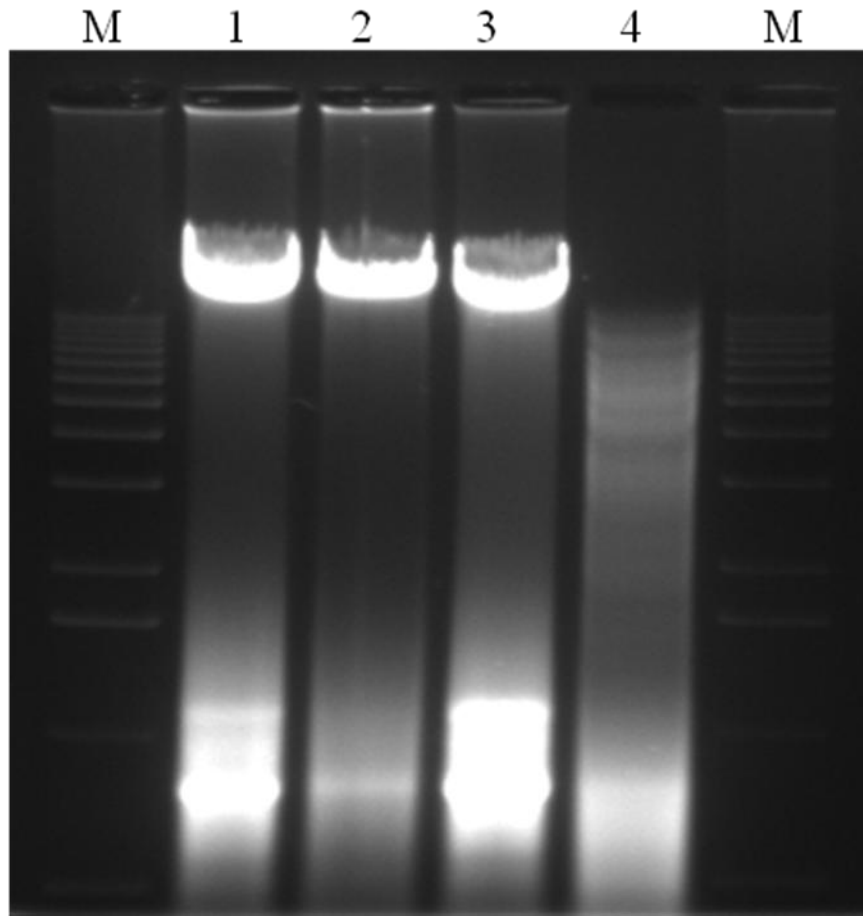
Supplementary Figures and Tables

Supplementary Table S1 Up-regulated and down-regulated genes in the hemHI deletion mutant as compared to the parental strain of *Shewanella loihica* PV-4

Function	Gene	Log2R	Z-score	Annotation
Iron uptake	Shew_3097	7.29	9.42	TonB-dependent siderophore receptor
	Shew_0861	7.04	7.67	extracellular solute-binding protein
	Shew_3841	7.69	5.15	TonB-dependent siderophore receptor
	Shew_3166	6.02	7.3	extracellular solute-binding protein
	Shew_1181	5.87	7.26	putative iron regulated membrane protein
	Shew_2517	4.45	5.46	ferrous iron transport protein B
	Shew_2518	4.19	4.18	ferrous iron transport protein A
	Shew_0552	4.47	3.11	Bacterioferritin associated ferredoxin
Cytochrome c	Shew_3112	-3.77	-5.09	flavocytochrome c
	Shew_0572	-3.51	-2.53	cytochrome c1
	Shew_2526	-3.19	-4.51	cytochrome c family protein
	Shew_2525	-2.98	-3.85	decaheme cytochrome c
	Shew_0918	-6.75	-5.25	decaheme cytochrome c
PPIX and heme biosynthesis	Shew_0678	-4.21	-7.71	protoporphyrinogen oxidase (hemG)
	Shew_0073	-4.38	-4.16	coproporphyrinogen III oxidase (hemN)
	Shew_2913	-3.54	-4.5	Glutamyl-tRNA reductase (hemA)
	Shew_3382	-3.4	-3.17	ALA dehydratase (hemB)
Flagella synthesis	Shew_1345	-7.20	-6.69	flagellar basal body rod protein FlgB
	Shew_1346	-6.76	-7.3	flagellar basal body rod protein FlgC
	Shew_1347	-6.73	-7.75	flagellar basal body rod modification protein
	Shew_1348	-6.12	-6.47	flagellar hook protein FlgE
	Shew_1350	-5.20	-7.19	flagellar basal body rod protein FlgG
	Shew_1352	-5.08	-7.69	flagellar basal body P-ring protein
	Shew_1354	-6.26	-8.17	flagellar hook-associated protein FlgK
	Shew_1355	-4.22	-5.58	flagellar hook-associated protein FlgL
Shew_1356	-5.79	-3.96	flagellin domain-containing protein	

Supplemental Table S2 The presence of *hemH1*, *hemH2*, *rpoE2*, *chrR* and *pgpD* orthologs in sequenced *Shewanella* genomes

Strains	Genes				
	<i>chrR</i>	<i>rpoE2</i>	<i>pgpD</i>	<i>hemH2</i>	<i>hemH1</i>
<i>S. oneidensis</i>	SO_1985	SO_1986	SO_3348	SO_3349	SO_2019
<i>S. denitrificans</i>	Sden_3373	Sden_3374	Sden_2679	Sden_2678	
<i>S. frigidimarina</i>	Sfri_2319	Sfri_2318	Sfri_2853	Sfri_2852	
<i>S. amazonensis</i>	Sama_2037	Sama_2036	Sama_2474		Sama_1316
<i>S. baltica OS155</i>	Sbal_2598	Sbal_2597	Sbal_3022	Sbal_3021	Sbal_2545
<i>S. baltica OS185</i>	Shew185_2637	Shew185_2636	Shew185_3037	Shew185_3036	Shew185_2583
<i>S. baltica OS195</i>	Sbal195_2712	Sbal195_2711	Sbal195_3180	Sbal195_3179	Sbal195_2660
<i>S. baltica OS223</i>	Sbal223_1748	Sbal223_1749	Sbal223_1341	Sbal223_1342	Sbal223_1801
<i>S. baltica OS678</i>	Sbal678_2717	Sbal678_2716	Sbal678_3186	Sbal678_3185	Sbal678_2663
<i>S. baltica OS117</i>	Sbal117_2734	Sbal175_1733	Sbal175_1323	Sbal175_1324	Sbal117_2682
<i>S. baltica BA175</i>	Sbal175_1732	Sbal175_1733	Sbal175_1323	Sbal175_1324	Sbal175_1783
<i>S. loihica</i>	Shew_1477	Shew_1476		Shew_2741	Shew_2229
<i>S. putrefaciens</i> CN-32	Sputcn32_2323	Sputcn32_2322	Sputcn32_2684	Sputcn32_2683	Sputcn32_2296
<i>S. putrefaciens 200</i>	Sput200_2349	Sput200_2348	Sput200_2821	Sput200_2820	Sput200_2322
<i>S. sediminis</i>				Ssed_1235	Ssed_2846
<i>S. pealeana</i>				Spea_1130	Spea_1529
<i>S. sp. MR-4</i>	Shewmr4_2276	Shewmr4_2275	Shewmr4_1196	Shewmr4_1197	Shewmr4_2248
<i>S. sp. MR-7</i>	Shewmr7_2348	Shewmr7_2347	Shewmr7_1267	Shewmr7_1268	Shewmr7_2320
<i>S. sp. ANA-3</i>	Shewana3_2466	Shewana3_2465	Shewana3_1197	Shewana3_1198	Shewana3_2440
<i>S. sp. W3-18-1</i>	Sputw3181_1685	Sputw3181_1686	Sputw3181_1327	Sputw3181_1328	Sputw3181_1712
<i>S. halifaxensis</i>				Shal_1175	Shal_1599
<i>S. woodyi</i>	Swoo_2779	Swoo_2780		Swoo_1335	
<i>S. piezotolerans</i>				swp_3670	swp_1736
<i>S. violacea</i>					SVI_2775



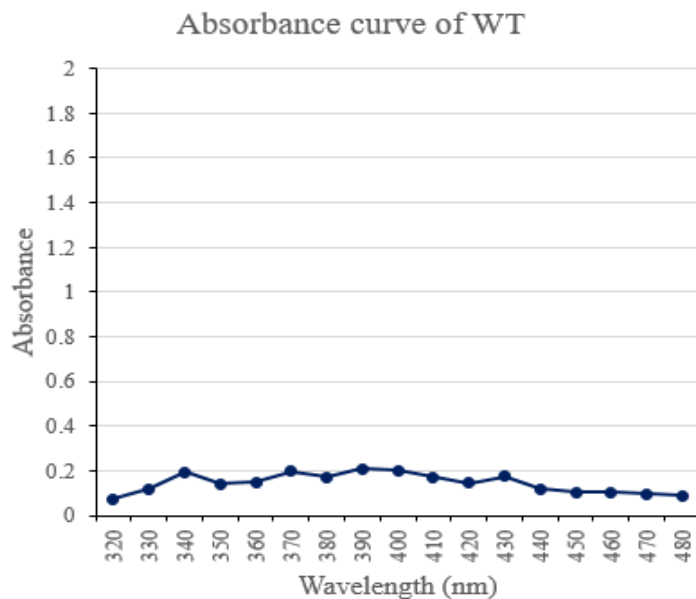
Supplementary Figure S1 PstI restriction digestion of chromosomal DNAs of the wild-type PV-4 strain (lane 2), and PV-4 Δ *pstI* Δ *pstM* strain (lane 4). M: 1 kb DNA markers; lanes 1 and 3 are the undigested chromosomal DNAs used as control. The chromosomal DNA of the wild-type PV-4 strain could not be digested by a commercial PstI endonuclease probably due to methylation of recognition sites. To facilitate genetic manipulation, we deleted the putative PstI-like endonuclease (designated PstI, encoded by the Shew_0993 locus) and DNA methylase (designated PstM, Shew_0992) genes in the PV-4 strain, resulting in the successful digestion of chromosomal DNA of the resultant PV-4 Δ *pstI* Δ *pstM* (used as the parental strain of PV-4 hereafter) by the PstI endonuclease. These results confirmed that PstM mediated methylation of chromosomal DNA of PV-4.

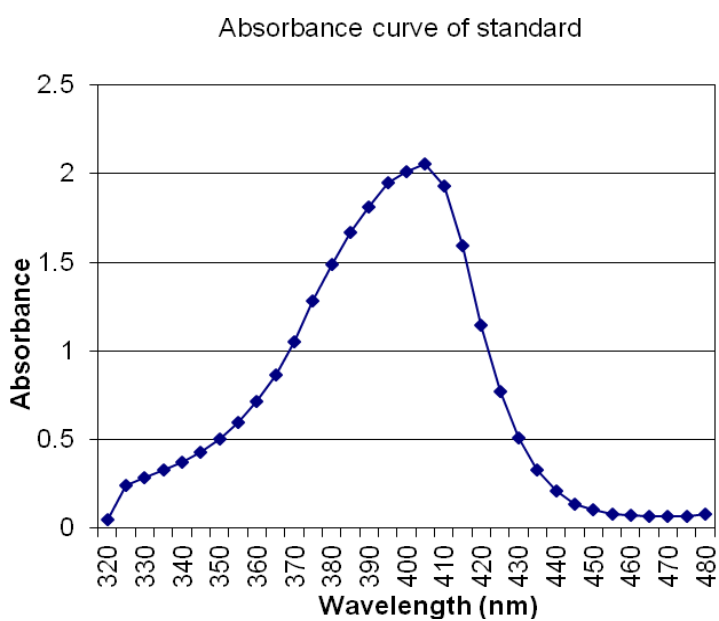
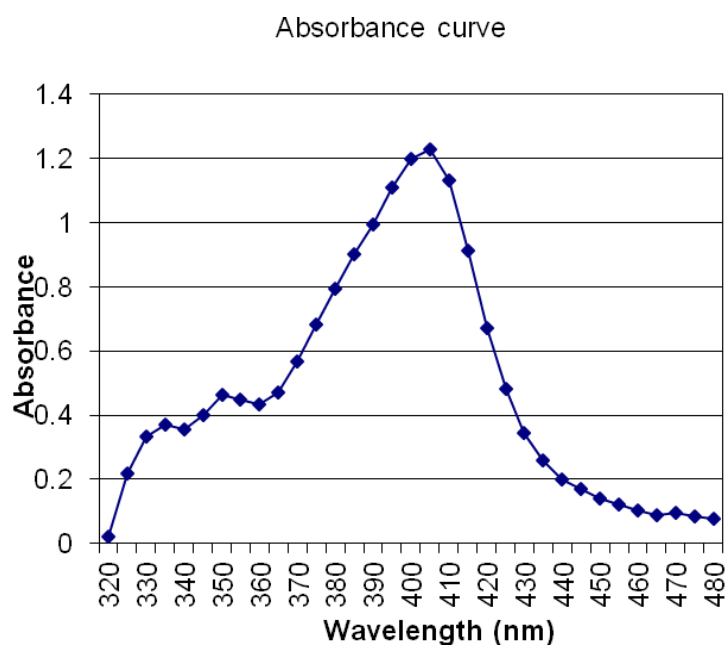
Start codon

TTGAATAAAGTCGACAAC**TA**TCTCCCGCTAAGCTTGGTGTCTTGCTGGTTAATCTCGG
CACACCCGATACACCAACGCCTAAAGATGTAAGCAGTTCTTAAAACAGTTTCTCAGC
GACCCGCGCGTGGTCGATCTAAATCCCTGGATCTGGAACCCATACTCAATGGCATCAT
ATTAAATACGCGCCCCAAGGCCGTAGCCAAGCTCTATGAGTCTATCTGGTGGCCCGAGG
GCTCGCCTCTGATGGTGATCAGTGAGCGCCAACGTGAAGCGCTGAGTGCGATTCTAAA
AGCGCGTCATGGTAGCGATATCCGGTGGAGTTGGGGATGAGTTATGGCAATCCCTCAC
TGTCGTGCGGGGATAGACAAGCTGGTGGCTCAGGGTGTGAGCGCCTGGTGGTGCTGCC
GCTATATCCTCAGTATTCCTGCTCCACCGTGGCACCGGTATTCGATGCCATCGCCAGTGA
CTATAAGGGCAGACGCAACTATCCTGAGACCCGCTTCAGTAAAGAGTATTTGAGCAC
CCGGCCTATATCGCGGCGCTGGCAGGTTCTGTGCGGCGTCATTGGCAAGATAAGGGCC
AGGGCGATTGCCTCTTGATGTCCTTTCATGGCGTGCCCTGCGT**TA**TGTGACCGAGGGT
GACCCCTATCAGCGTCAGTGTCAACGTACTGCCGAGCTGCTGGCCGCGGCCTTGGGGC
TTACAGAGTCTCAGTGGCGCCTGTGTTTTCAATCGAAATTTGGTAAGGAGGAGTGGCT
GACGCCGGAACGGATGCGCTGCTCGAGAGTTTGCCGGGTAAAGGGGTTAAGCGCGT
GGATATTCTCTGTCCGGCCTTTGCCGTGGATTGTTTGGAGACGCTTGAAGAGATCTCCA
TTGGCGGCAAGGAGAGCTTTATCGAGGCCGGCGGTGAGGACTATCATTTCATCCCTTGT
TTGAACGAGGATGAAGCGCATATGCAGCTGCTCGCGGACTTGGTCGATCAACAGGCGG
CGGGCTGGCTTAAATCT**TAA**

Stop codon

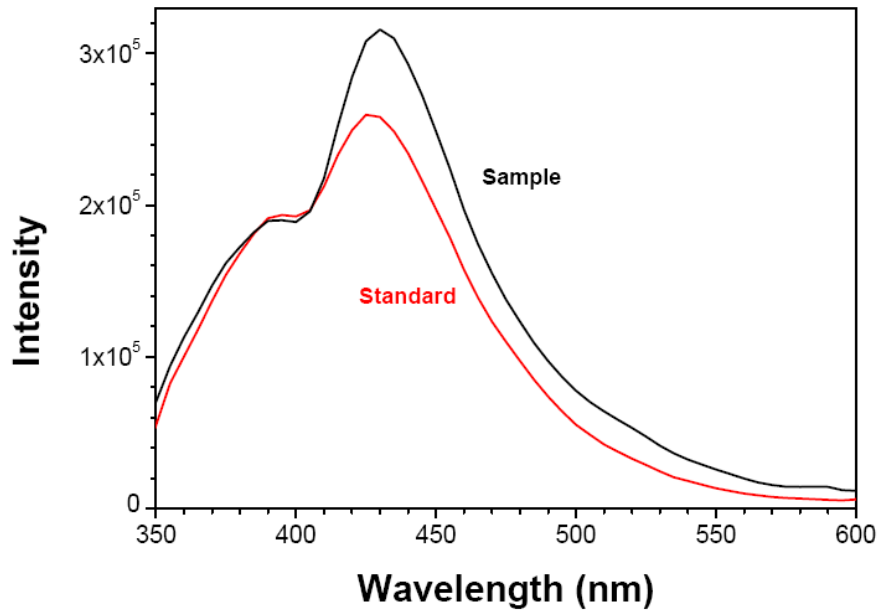
Supplementary Figure S2 Mapping of insertional sites of transposon in the red pigment-overproducing transposon mutants of *Shewanella loihica* PV-4. The open reading frame of *hemH1* (locus Shew_2229) is shown. The *mariner* transposon recognizes the dinucleotide TA for insertion and the insertional sites of two transposon insertional mutants are highlighted.





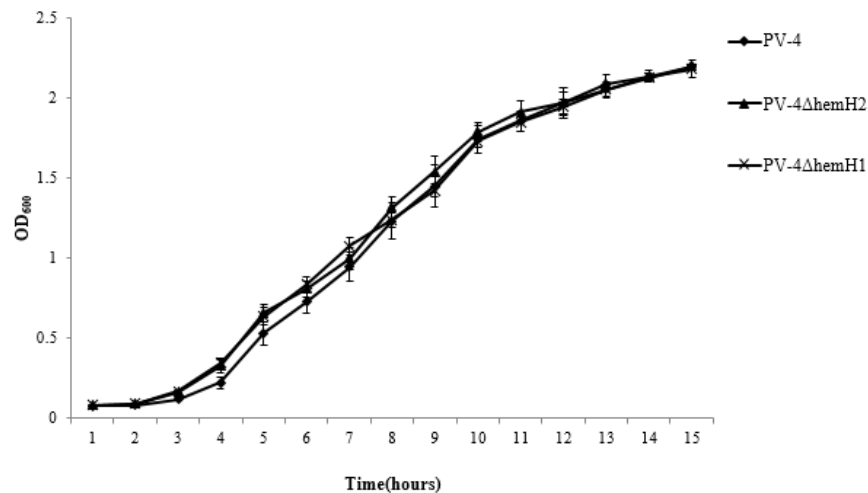
Supplementary Figure S3 Ultraviolet-visible spectrograms of the bacterial extract (the wildtype and the *hemHI* mutant) and PPIX standard. Absorbance was measured at every 5 or 10 nanometers (nm).

Fluorescence Spectra of PPIX

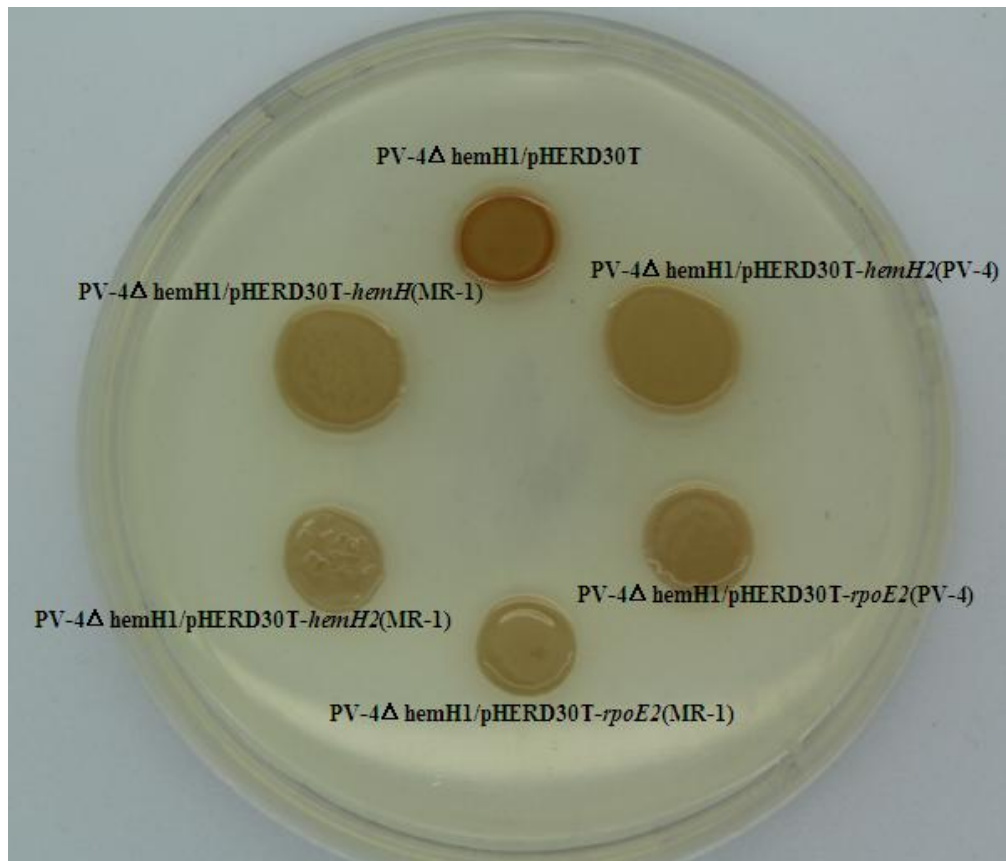


Supplementary Figure S4 The fluorescence spectra of the PPIX standard and the extracted sample showing their similarity.

the growth of PV-4 and mutants



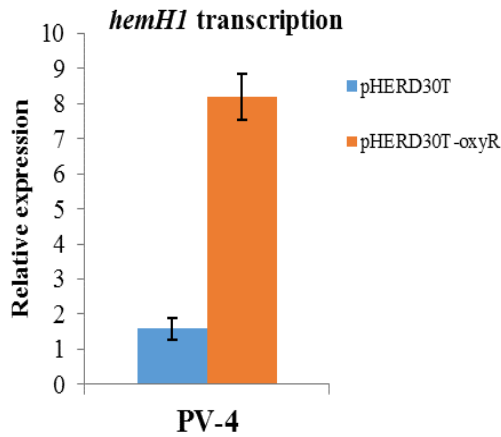
Supplementary Figure S5 Bacterial growth (optical density at 600 nm) of PV-4 and the PV-4ΔhemH1 and PV-4ΔhemH2 mutants in the LB broth. Culture tubes were incubated at 28 °C, 220n/min.



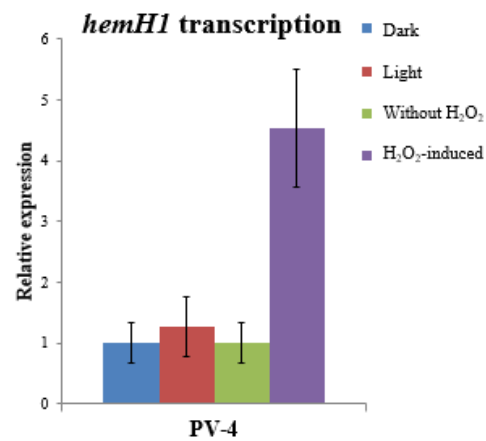
Supplementary Figure S6 Overexpression of plasmid borne *hemH1*, *hemH2* or *rpoE2* genes from PV-4 and MR-1 suppressed the PPIX-overproducing phenotype of the PV4Δ*hemH1* strain.

ClustalW2 to identify the promoter motifs and the putative OxyR (encoded by *shewShew_1035*)-recognized motif. The shadowed motifs are supposedly predicted OxyR-recognized elements, and the underlined motifs are RpoD-recognized elements.

(a)

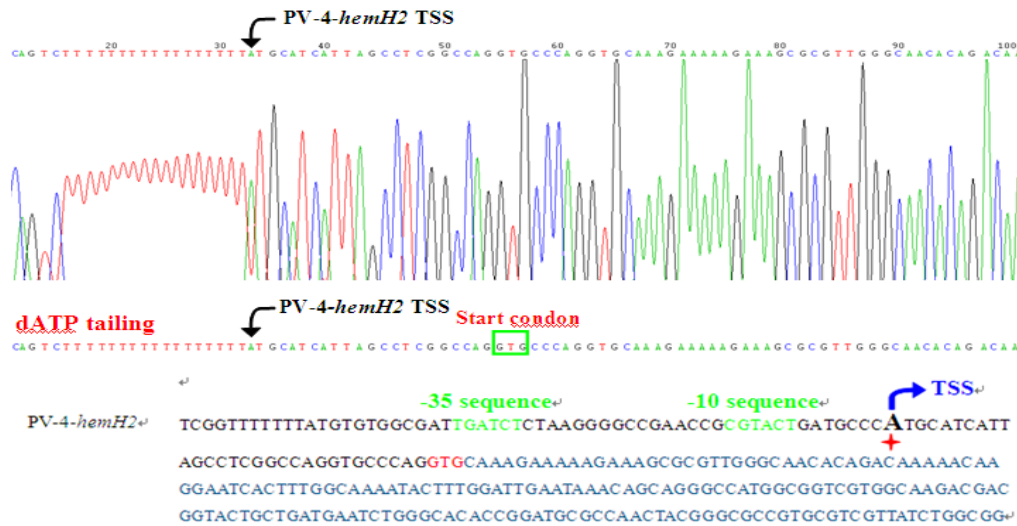


(b)

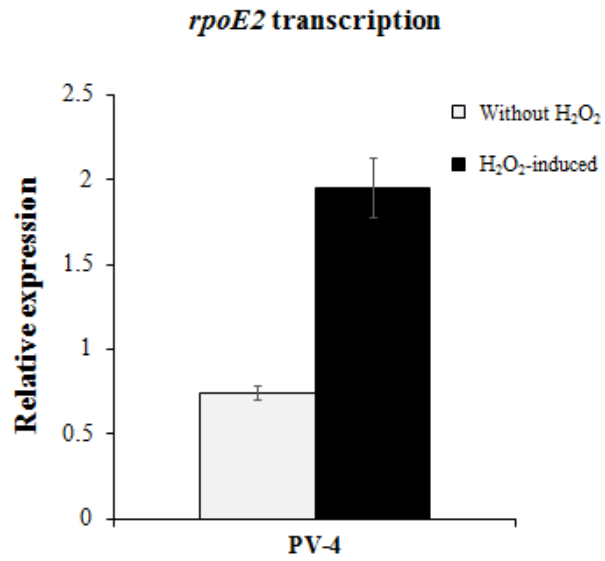


Supplementary Figure S8 (a) Expression of the *oxyR* gene *in trans* activated expression of *hemH1* in the *S. loihica* PV-4 strain. (b) Effects of hydrogen peroxide (H₂O₂) and visible light on the transcription of the *hemH1* in the *S. loihica* PV-4.

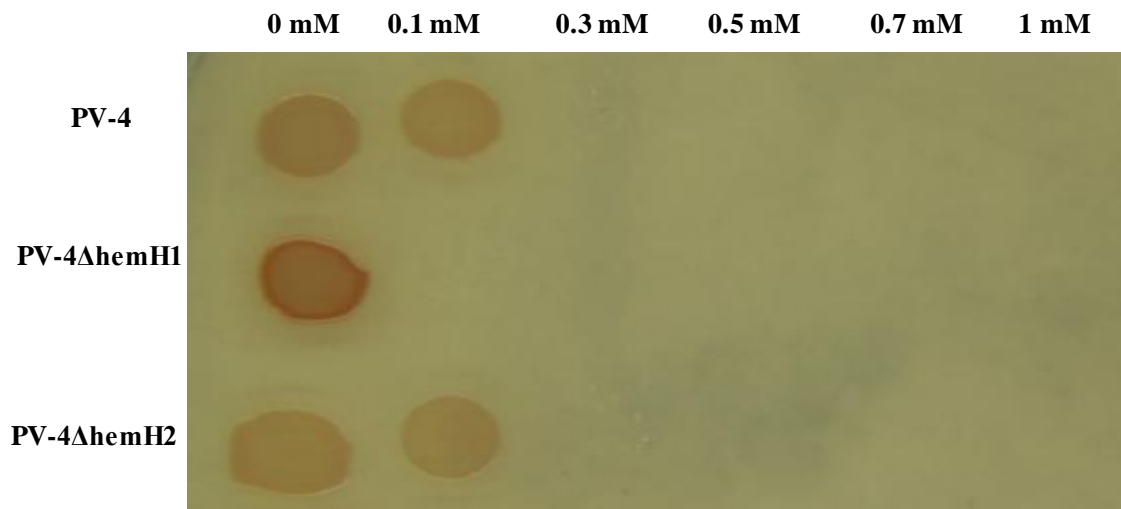
PV-4-hemH2

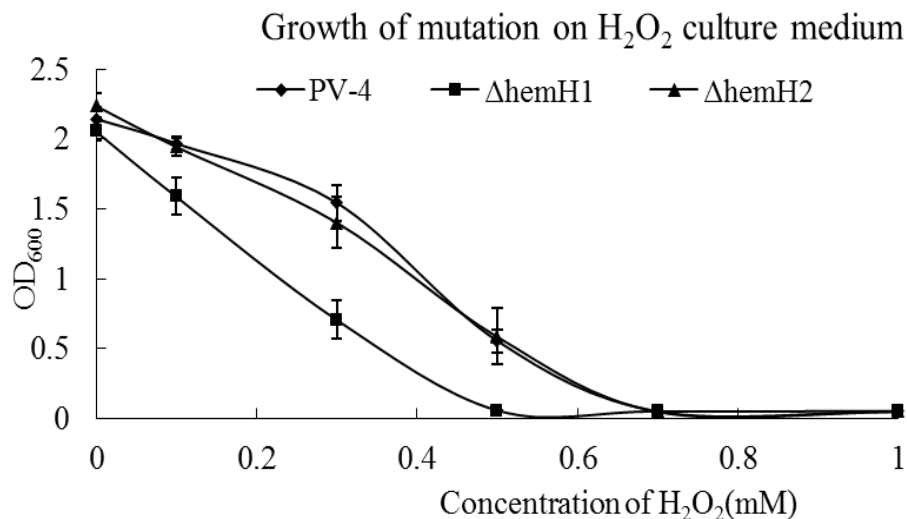


Supplementary Figure S9 Primer extension analysis of transcriptional start site (TSS) of *hemH2* (Shew_1140) in the *S. loihica* PV-4 strain.



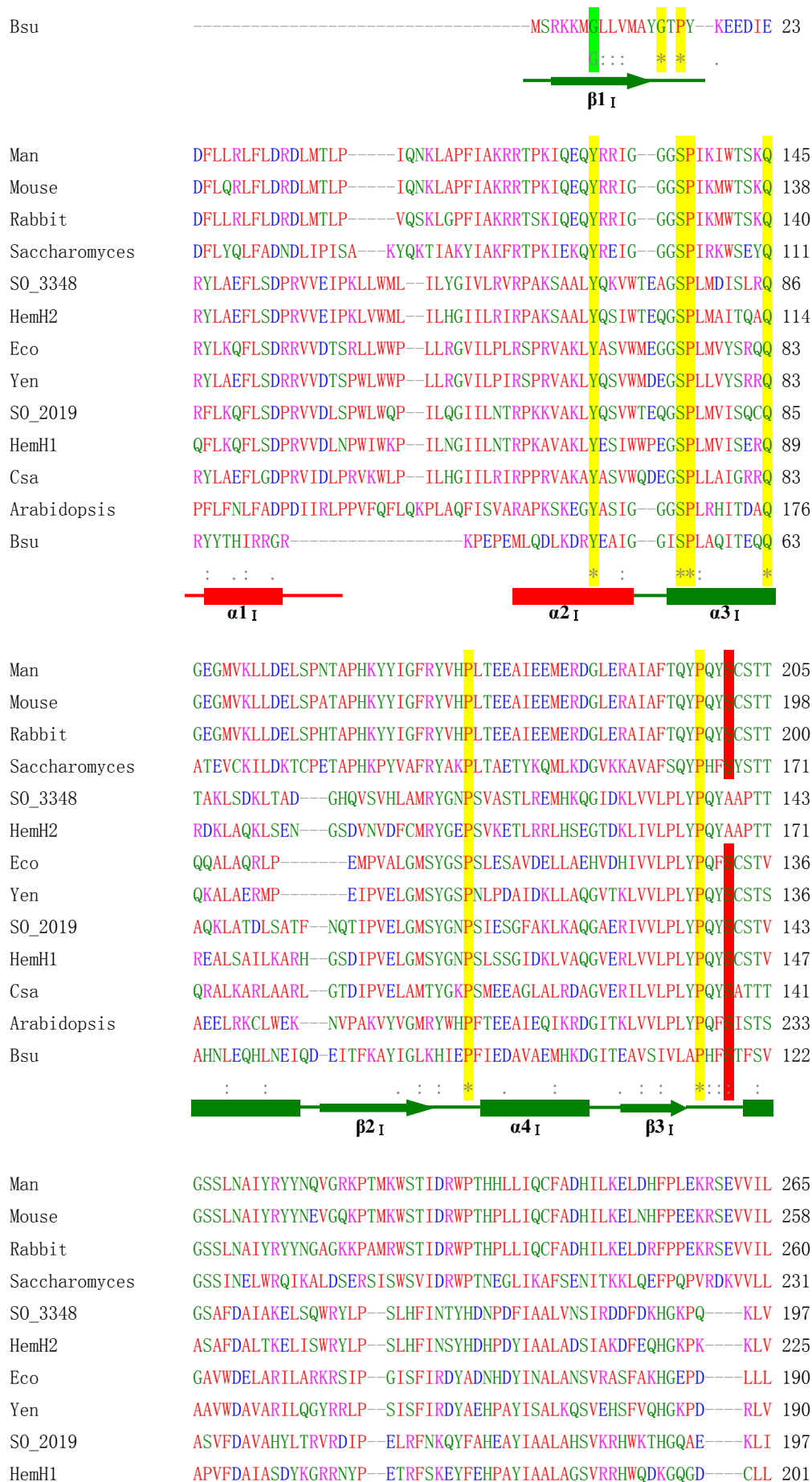
Supplementary Figure S10 Expression of *rpoE2* in PV-4 under different H₂O₂-induced oxidative stress condition.



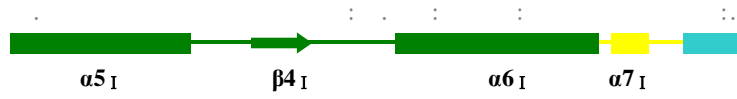


Supplementary Figure S11 Effects of hydrogen peroxide (H₂O₂) on the bacterial growth of the *Shewanella loihica* strains. Wild type PV-4 strain and the *hemH1* and *hemH2* mutants were grown in the LB broth containing 0, 0.1, 0.3, 0.5, 0.7 and 1 mM of hydrogen peroxide and incubated at 28 °C for 18 hrs.

Man	-----M	RS	LG	AN	MAA	AL	RA	AG	VLL	RD	PL	AS	SS	WR	VC	QP	WR	35																																										
Mouse	-----M	LS	SA	AN	MAA	AL	RA	AG	ALL	RE	PL	VH	GS	SR	AC	QP	WR	35																																										
Rabbit	-----M	LS	AG	TN	MAA	AL	RA	AG	AL	FR	CP	SV	HG	SR	AH	QP	WR	35																																										
Saccharomyces	-----M	LS	R	-----T	IR	TQ	GS	FL	RR	SQ	LT	IT	-----	-----	-----	-----	-----	22																																										
S0_3348	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----																																										
HemH2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----																																										
Eco	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----																																										
Yen	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----																																										
S0_2019	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----																																										
HemH1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----																																										
Csa	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----																																										
Arabidopsis	M	N	C	P	A	M	T	A	S	P	S	S	S	S	S	S	S	S	Y	S	T	F	R	P	P	P	L	L	P	Q	L	S	N	D	S	Q	R	S	V	V	M	H	C	T	R	L	P	F	E	A	F	A	A	T	S	S	N	R	L	60
Bsu	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----																																									
Man	W	K	S	G	A	-----A	A	A	A	V	T	T	E	T	A	Q	H	A	Q	G	A	K	P	Q	V	Q	P	Q	K	R	Y	E	S	N	I	R	K	P	K	T	-----I	L	M	L	N	M	G	G	P	E	-----T	L	G	D	V	H	92			
Mouse	C	Q	S	G	-----A	A	V	A	A	T	T	E	K	V	H	H	A	K	T	K	P	Q	A	Q	P	E	-----R	K	P	K	T	-----I	L	M	L	N	M	G	G	P	E	-----T	L	G	E	V	Q	85												
Rabbit	C	Q	S	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	T	T	Q	R	A	Q	S	T	K	P	Q	V	Q	P	E	-----R	K	P	K	T	-----I	L	M	L	N	M	G	G	P	E	-----T	L	G	E	V	H	87					
Saccharomyces	-----S	F	S	V	T	F	N	M	Q	N	A	Q	-----K	R	S	P	T	G	I	V	L	M	N	M	G	G	P	S	-----K	V	E	E	T	Y	56																									
S0_3348	-----M	G	H	A	A	R	G	K	V	-----V	L	L	L	N	L	G	T	P	D	A	P	T	S	A	V	R	28																																	
HemH2	---M	Q	R	K	R	K	R	V	-----Q	H	R	Q	K	Q	G	I	T	L	A	K	Y	F	G	L	N	K	-----Q	Q	G	H	G	R	G	K	T	-----V	L	L	M	L	N	L	G	T	P	D	A	P	T	G	A	V	R	56						
Eco	-----M	R	Q	T	K	-----I	L	L	N	L	G	T	P	D	A	P	T	P	E	A	V	K	25																																					
Yen	-----M	K	Q	S	K	L	-----V	L	M	V	N	L	G	T	P	D	A	P	T	Q	A	V	K	25																																				
S0_2019	-----M	T	S	P	S	P	A	-----F	V	L	L	V	N	L	G	T	P	D	E	P	T	P	K	A	V	K	27																																	
HemH1	-----M	N	K	V	D	N	-----S	P	A	K	L	-----V	L	L	V	N	L	G	T	P	D	T	P	K	D	V	K	31																																
Csa	-----M	G	S	A	P	F	-----V	M	L	V	N	L	G	T	P	E	A	P	T	S	A	V	R	25																																				
Arabidopsis	G	K	H	S	L	P	L	R	A	A	L	V	T	S	N	P	L	N	I	S	S	S	S	V	I	S	D	A	I	S	S	S	V	I	T	D	D	A	K	I	-----V	L	L	L	N	L	G	G	P	E	-----T	L	D	D	V	Q	118			



Csa GAVFDRLARALSPCPHLP--ELRFVVDYHDHPAYIEALAESIREHWETHGRQP---RLL 195
 Arabidopsis GSSLRLLERIFREDEYLVNMQHTVIPSWYQREGYIKAMANLIQSELGKFGSPNQ--VVIF 291
 Bsu QSYNKRAKEEAELKGLT---ITSVESWYDEPKFVTVWDRVKETYASMPEDERENAMLI 179



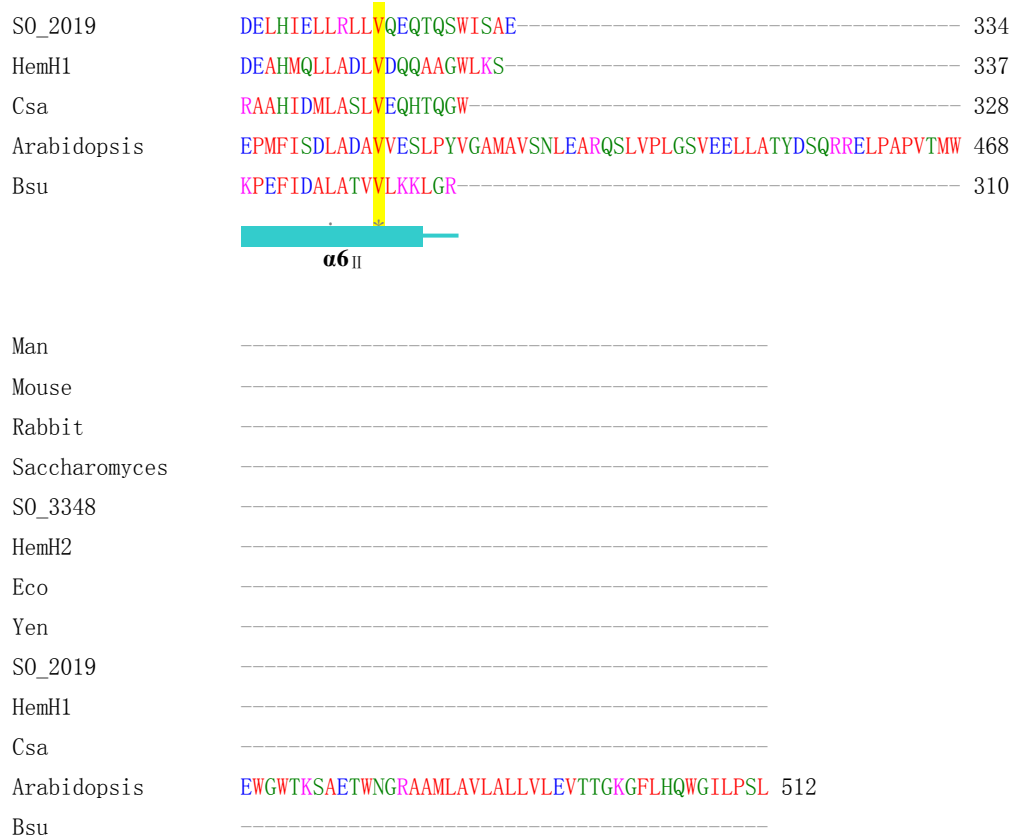
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 Mouse FSAHSLPMSVVNR-GDPYPQEVGATVHKVMEKLGYP--NPYRLVWQSK-VGPVPWLG- 312
 Rabbit FSAHSLPMSVVNR-GDPYPQEVGATVHRVMERLGY- --NPYRLVWQSK-VGMPWPWLG- 314
 Saccharomyces FSAHSLPMDVVNT-GDAYPAEVAATVYNIMQKLFK--NPYRLVWQSQ-VGPKPWLGA- 285
 SO_3348 LSYHGMPERNLHL-GDPYFCMKTTRLVAEQLGLS--KDEFAITFQSR-FGKAKWLQP- 252
 HemH2 LSYHGMPERNLNL-GDPYCLCQKTTRLVVERLGLT--DDYITTFQSR-FGKAKWLGP- 280
 Eco LSYHGIPQRYADE-GDPYQRCRTTRELASALGMA--PEKVMMTFQSR-FGREPWLMP- 245
 Yen LSFHGIPKRYAQL-GDDYQRCEDTSRALRAEIALP--AEQIMMTYQSR-FGREPWLTP- 245
 SO_2019 LSFHGIPRYATE-GDPYEQCRTTAKLLAALGLT--DGQWQVCFQSR-FGKEEWLTP- 252
 HemH1 MSFHGVPLRYVTE-GDPYQRCQRTAELLAALGLT--ESQWRLCFQSK-FGKEEWLTP- 256
 Csa FSYHGIPKRYAEA-GDPYPRHCETTSRLVAEALGLE--PEAWQQTYQSR-FGREEWLKP- 250
 Arabidopsis FSAHGVPPLAYVEEAGDPYKAEMEECVDLIMEELDKRKITNAYTLAYQSR-VGPVWLP- 349
 Bsu VSAHSLPEKIKEF-GDPYDQLHESAKLIAEGAGVS---EYAVGWQSEGNTDPWLGPD 234



Man QTDESIKGLCERGRKNILLVPIAFTSDHIETLYELDIEYSQVLAKECGVENIRRAESLNG 379
 Mouse QTDEAIKGLCERGRKNILLVPIAFTSDHIETLYELDIEYSQVLAQKCGAENIRRAESLNG 372
 Rabbit QTDETIKGLCERGRKNILLVPIAFTSDHIETLYELDIEYSQVLAKECGVENIRRAESLNG 374
 Saccharomyces QTAEIAEFLGPK-VDGLMFIPIAFTSDHIETLHEIDLG---VIGESEYKDKFKRCESLNG 341
 SO_3348 YTDATMAALPSQGVDRVAIVCPAFSADCLETLEEIVGENGHIFTHAGGK-FRYIPALND 311
 HemH2 YTDASLEALAKEGVDDVAIVCPAFSADCLETLEEIEHENRDVFTQAGGSE-YRYIPCLND 339
 Eco YTDETLKMLGEKGVGHIQVMCPGFAADCLETLEEIAEQNREVFLGAGGK-YEYIPALNA 304
 Yen YTDETLKSLPSQGVKHIQLICPGFSADCLETLEEIKEQNREIFLHAGGK-FEYIPALND 304
 SO_2019 YADELLADLPRQGVKSVDVICPAFATDCLETLEEISIGAKETFLHAGGEE-YHFIPCLND 311
 HemH1 ATDALLESPLGKGVKRVDIRCPAFVDCLETLEEISIGKESFIEAGGED-YHFIPCLNE 315
 Csa YTDDTLKAWGVEGLEVDVISPAFAADCLETLEELEVENRGYFTEAGGDD-YRYIPALND 309
 Arabidopsis YTEEAITELGKKGVENLLAVPISFVSEHIETLEEIDVEYKELALKSGIKN-WGRVPALGT 408
 Bsu VQDLTRDLFEQKGYQAFVYVPVGFVADHLEVLYDNDYECKVVTDDIGASY--YRPEMPNA 292



Man NPLFSKALADLVHSHIQSNELCSKQLTLSCPLCVN--PVCRETKSFFTSQQL----- 429
 Mouse NPLFSKALADLVHSHIQSNKLCSTQLSLNCPVCN--PVCRTKSFFTSQQL----- 422
 Rabbit NPLFSKALADLVHAHIQSDELCSKQWTLGCPLCVN--PICRETKSFFTNQRL----- 424
 Saccharomyces NQTFIEGMADLVKSHLQSNQLYSNQLPLDFALGKSNPDKLSLVFGNHEST----- 393
 SO_3348 NDDHIAMMANLVKPYL----- 327
 HemH2 QELHIQMMVNLVVRPYL----- 355
 Eco TPEHIEMMANLVAAAYR----- 320
 Yen DKGHIDLLEQLVRDHLSC----- 322



Supplementary Figure S12 Alignment of ferrochelatase amino acid sequences of various organism. Conserved regions of the sequence are highlighted in yellow, and minor differences are boxed in green (Thr and Gly) and red (Ser and Ala). Secondary structure elements are shown as determined from the *B. subtilis* enzyme (1). The alignment was generated by ClustalW2. The aligned sequences were from ferrochelatase amino acid sequences of a variety of organisms: Eco (*Escherichia coli*), Yen (*Yersinia enterocolitica*), Csa (*Cucumis sativus*), man, mouse, Rabbit (*Oryctolagus cuniculus*), Saccharomyces (*Saccharomyces cerevisiae*), Arabidopsis (*Arabidopsis thaliana*), Bsu (*B. subtilis*), the two HemH paralogues of the *S. loihica* PV-4 and the *S. oneidensis* MR-1.

REFERENCES

1. Al-Karadaghi S, Hansson M, Nikonov S, Jönsson B, Hederstedt L. 1997. Crystal structure of ferrochelatase: the terminal enzyme in heme biosynthesis. Structure 5: 1501-1510.