**Additional file 1**

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**Differential gene content and gene expression for bacterial evolution and speciation of Shewanella in terms of biosynthesis of heme and heme-requiring proteins**

Jingcheng Dai1, Yaqi Liu1, Shuangyuan Liu1,Shuyang Li1, Na Gao1, Jing Wang1, Jizhong Zhou2,3, Dongru Qiu1\*

1 Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China and University of Chinese Academy of Sciences, Beijing 100049, China

2 Institute for Environmental Genomics, and Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019, USA

3 Earth Science Division,Lawrence Berkeley National Laboratory, Berkeley, CA 94270, USA

**\*Correspondence to: Dongru Qiu, PhD**

Institute of Hydrobiology, Chinese Academy of Sciences

Wuhan 430072, Hubei Province, China

Phone: 0086-27-68780215; Fax: 0086-27-68780123

E-mail: qiu@ihb.ac.cn

**Table S1** The heme proteins of *S. oneidensis* MR-1.

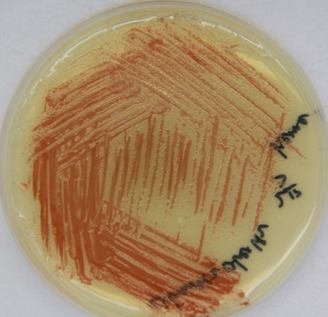
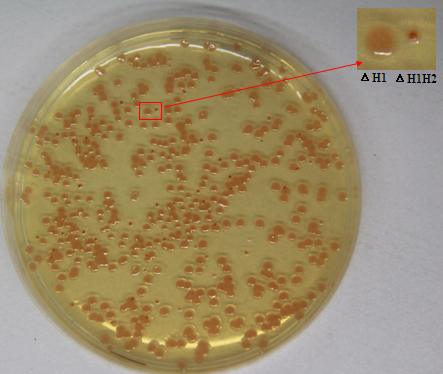
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ID** | **Gene** | **Protein** | **Biological process** | **Prostetic group** |
| SO\_1070 | *katB* | Catalase B | H2O2 scavenging | Protoheme IX, heme D? |
| SO\_4405 | *katG1* | Catalase G | Protoheme IX, heme B |
| SO\_0725 | *katG2* | Catalase G | Protoheme IX, heme B |
| SO\_1776-SO\_1778 | *mtrABC* | Extracellular iron oxide respiratory  protein | Electron transfer | Protoheme IX, heme B |
| SO\_1779 | *omcA* | Protoheme IX, heme B |
| SO\_1780-SO\_1782 | *mtrDEF* | Extracellular respiratory  protein | Protoheme IX, heme B |
| SO\_3285-SO\_3286 | *cydAB* | Cytochrome bd oxidase-II | Protoheme IX, (heme D?) |
| SO\_1427-SO\_1428 | *dmsEF* | Periplasmic decaheme cytochrome c | Protoheme IX, heme B |
| SO\_4606-SO\_4607 | *coxBA* | Cytochrome C oxidase | Protoheme IX, heme A |
| SO\_4609 | *coxC* | Cytochrome C oxidase | Protoheme IX, heme A |
| SO\_4613-SO\_4614 | *ctaA-ctaB* | Cytochrome C oxidase | Protoheme IX, heme A |
| SO\_0790 | *fccA* | Fumarate reductase flavoprotein | Protoheme IX, heme B? |
| SO\_2361-SO\_2364 | *ccoPQON* | Cytochrome C oxidase | Protoheme IX, heme A |
| SO\_0101-SO\_0103 | *fdnGHI* | Formate dehydrogenase-O | Protoheme IX, heme B/D |
| SO\_1233 | *torC* | Trimethylamine-N-oxide reductase | Protoheme IX, heme B |
| SO\_4591 | *cymA* | Tetraheme cytochrome c | Protoheme IX, heme B |
| SO\_1927-SO\_1930 | *sdhCDAB* | Succinate dehydrogenase | Electron transfer, TCA cycle | Protoheme IX, heme B |
| SO\_1111-SO\_1112 | *bfr* | Bacterioferritin | Iron storage | Protoheme IX, heme B |
| SO\_2178 | *ccpA* | Cytochrome C peroxidase | Unknow | Protoheme IX, heme B |
| SO\_0740 | *unknow* | Dyp-type heme-dependent peroxidase | Unknow | Protoheme IX, heme B |
| SO\_3980 | *nrfA* | Nitrite reductase | Anaerobic respiration | \*Siroheme |
| SO\_4568 | *nrfD* |
| SO\_0845-SO\_0849 | *napBHGAD* | Periplasmic nitrate reductase | Anaerobic respiration | Protoheme IX, heme B |
| SO\_1783-SO\_1784 | *feoAB* | Ferrous iron transporter | Uptake of ferrous iron under conditions of iron limitation and low pH | Protoheme IX, heme B |
| SO\_3737-SO\_3738 | *cysIJ* | Sulfite reductase | Sulfate assimilation | \*Siroheme |

\*Siroheme synthesis departs from the pathway of heme synthesis after the HemD step and does not involve coproporphyrinogen III oxidase (HemN/HemF) or ferrochelatase (HemH).

**Table S2** Iron acquisition and metabolism function between PV-4 and MR-1.

|  |  |  |
| --- | --- | --- |
| Strains | Function | Expressed protein |
| MR-1 | Siderophore assembly kit | Siderophore biosynthesis protein, monooxygenase |
| MR-1 | Siderophore assembly kit | Siderophore synthetase component, ligase |
| MR-1 | Siderophore assembly kit | Siderophore synthetase small component, acetyltransferase |
| MR-1 | Heme, hemin uptake and utilization systems in Gram-Negatives | ABC-type hemin transport system, ATPase component |
| MR-1 | Heme, hemin uptake and utilization systems in Gram-Negatives | Biopolymer transport protein ExbD1 |
| MR-1 | Heme, hemin uptake and utilization systems in Gram-Negatives | Hemin ABC transporter, permease protein |
| MR-1 | Heme, hemin uptake and utilization systems in Gram-Negatives | Periplasmic hemin-binding protein |
| MR-1 | Heme, hemin uptake and utilization systems in Gram-Negatives | Putative heme iron utilization protein |
| MR-1 | Heme, hemin uptake and utilization systems in Gram-Negatives | phosphate oxidase-related putative heme iron utilization protein |
| MR-1 | Heme, hemin uptake and utilization systems in Gram-Negatives | TonB-dependent hemin , ferrichrome receptor |
| MR-1 | Heme, hemin uptake and utilization systems in Gram-Negatives | Transcriptional regulator LuxT |
| MR-1 | Transport of Iron | Iron-uptake factor PiuC |
| PV-4 | Siderophore Aerobactin | Aerobactin siderophore receptor IutA |
| PV-4 | ABC transporter [iron.B12.siderophore.hemin] | ABC transporter (iron.B12.siderophore.hemin) , ATP-binding component |
| PV-4 | Transport of Iron | Iron-regulated protein A precursor |
| PV-4 | Transport of Iron | Iron-uptake factor PiuB |
| PV-4 and MR-1 | Encapsulating protein for DyP-type peroxidase and ferritin-like protein oligomers | Predicted dye-decolorizing peroxidase (DyP), YfeX-like subgroup |
| PV-4 and MR-1 | Hemin transport system | Ferric siderophore transport system, periplasmic binding protein TonB |
| PV-4 and MR-1 | Hemin transport system | Outer membrane receptor proteins, mostly Fe transport |
| PV-4 and MR-1 | Iron acquisition in Streptococcus | Ferric iron ABC transporter, ATP-binding protein |
| PV-4 and MR-1 | Iron acquisition in Streptococcus | Ferric iron ABC transporter, iron-binding protein |
| PV-4 and MR-1 | Iron acquisition in Streptococcus | Ferric iron ABC transporter, permease protein |
| PV-4 and MR-1 | Transport of Iron | Ferric iron ABC transporter, ATP-binding protein |
| PV-4 and MR-1 | Transport of Iron | Ferric iron ABC transporter, iron-binding protein |
| PV-4 and MR-1 | Transport of Iron | Ferric iron ABC transporter, permease protein |
| PV-4 and MR-1 | Transport of Iron | Ferric uptake regulation protein FUR |
| PV-4 and MR-1 | Transport of Iron | Ferrichrome-iron receptor |
| PV-4 and MR-1 | Transport of Iron | Ferrous iron transport protein A |
| PV-4 and MR-1 | Transport of Iron | Ferrous iron transport protein B |

**Figure S1** the double deletion of *hemH1* and *hemH2* in MR-1 strain



**Figure S2** Ultraviolet-visible spectrograms of the bacterial extract(the wildtype and the *hemH1* mutant). Absorbance was measured at every 5 or 10 nanometers (nm).

**Figure S3** Alignment of promoter sequences upstream of loci SO\_3349-SO\_3348(*pgpD-hemH2*) of *S. oneidensis* MR-1 andShew\_1140(*hemH2*) in *S. loihica* PV-4. The putative RpoE2 recognizing motifs (-35 motif TGATCC/T and -10 motif CGTAA/CT) are highlighted in yellow.

CLUSTAL 2.1 multiple sequence alignment

SO\_3349 TCCTATTTTGTCATATGTTAAAGCAGCTTTTTGAAGCTGCTTTTTATCGCTTAGGGTAAT 60

SHEW\_1140 -CC--TCTTATTGCACTTTGAAAAAACC--------------------------GATAAT 31

\*\* \* \*\* \* \* \*\* \*\* \* \* \* \*\*\*\*

SO\_3349 AGATAACTCCTTGTAGCTTTTATTTTTTTATT-CAATATGATCCCTATCGTAGCAAGTTA 119

SHEW\_1140 ------CCCTTTATCGGTTTT-TTTATGTGTGGCGAT-TGATCTCTAAGGGGCCGAACCG 83

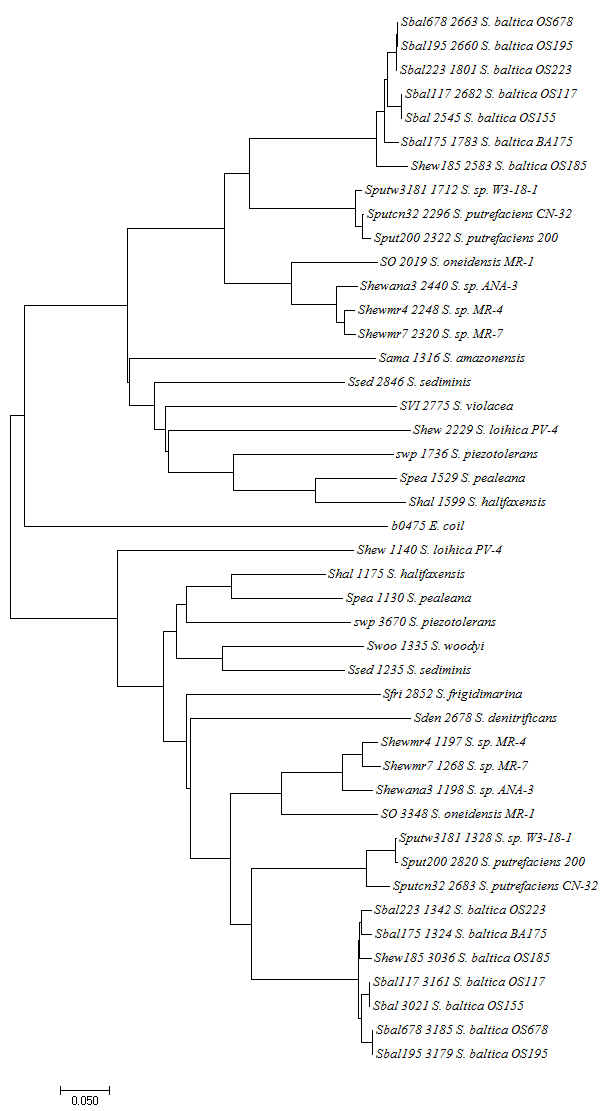
\* \* \*\* \* \* \*\*\*\* \*\*\* \* \* \* \* \*\* \*\*\*\*\* \*\*\* \* \* \*

SO\_3349 CGTAATAAGAATAAGTTCGCCTATCTTGGATCATTGAA-TGAGAAGGACATT--- 170

SHEW\_1140 CGTACTGA---------TGCCCA---TGCATCATTAGCCTCGGCCAGGTGCCCAG 126

\*\*\*\* \* \* \*\*\* \* \*\* \*\*\*\*\*\* \* \* \*

**Figure S4** Distance tree of *hemH* paralogues in *Shewanella* based on DNA sequence similarity. *E.coli hemH* used as reference. Clearly, the two *hemH* paralogues fall into two cluster, *hemH1* sequences form the upper gene cluster, while *hemH2* sequences form the lower cluster.



*hemH1*

*hemH2*