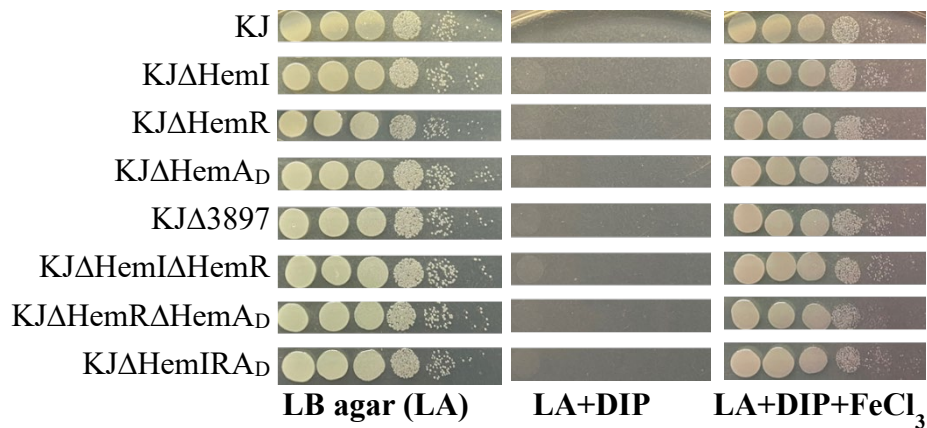


Fig. S1. Growth curve of parental strain KJΔEnt and its derived mutants grown under iron-depleted with hemin as sole iron source. An overnight bacterial culture was inoculated to LB broth supplemented with 50 μg/mL DIP and 150 μM hemin at an initial OD_{450 nm} of 0.15. Bacterial growth was monitored by recording the OD_{450 nm} for 24 h at intervals of 3 h. Graph is representative of at least three independent experiments.

(A)



(B)

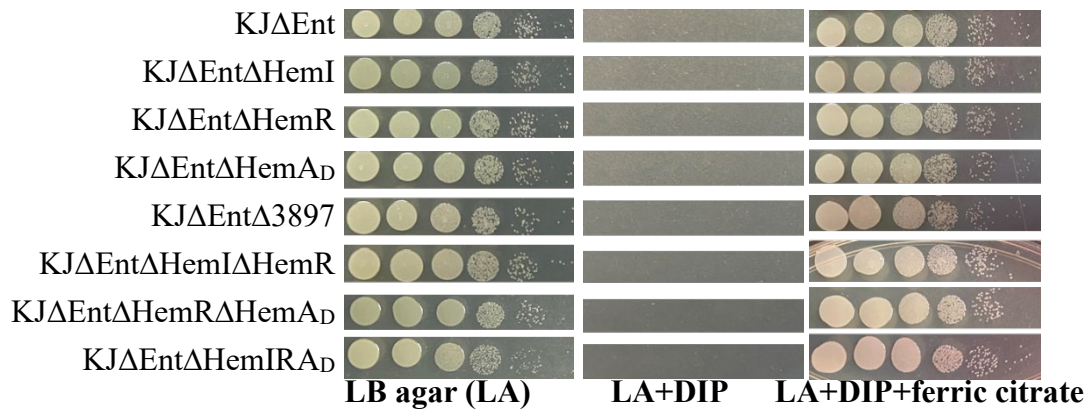


Fig. S2. Role of *smlt3897* and the *hemI-hemR-hemA_D* operon in the acquisition of ferric chloride and ferric citrate under iron-depleted conditions. Overnight cultures were inoculated into fresh LB broth at an initial OD₄₅₀ = 0.15 and incubated for 5 h at 37°C. Cultures were then adjusted to 2 × 10⁵ CFU/μL and subjected to 10-fold serial dilution. Five-microliter aliquots were spotted onto LB agar with or without the indicated supplements. Cell viability was assessed after 24 h at 37°C. Images are representative of at least three independent experiments. DIP, 2,2'-dipyridyl (50 μg/mL); FeCl₃, 35 μM; ferric citrate, 110 μM. (A) Role of *smlt3897* and *hemI-hemR-hemA_D* in ferric chloride utilization under iron-depleted conditions. (B) Role of *smlt3897* and *hemI-hemR-hemA_D* in ferric citrate utilization under iron-depleted conditions.

(A)

```
K279a, smlt3898 1 ATGACGCCGCGCCTGCGGCACCGATTACCTGACAGGTTCCCCATG 45
                  |||
KJ, hemAD      1 ATGACGCCGCGCCCCGCGGCACTGAATTACCTGACAGGTTCCCCATG 45
```

(B)

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K279a, Smlt3898 1 MTPRLRHRLPDRFPM 15
KJ, HemAD      1 MTPRPRH-LPDRFPM 15
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Fig. S3. Alignment of the 1-45 nucleotide sequences and their encoded peptide sequences between *smlt3898* of strain K279a and *hemA_D* of strain KJ. The stop codon in *hemA_D* is highlighted in gray. (A) Alignment of the 1-45 nucleotides between *smlt3898* (K279a) and *hemA_D* (KJ). (B) Alignment of the first 15 amino-acid residues between *smlt3898* (K279a) and *hemA_D* (KJ).

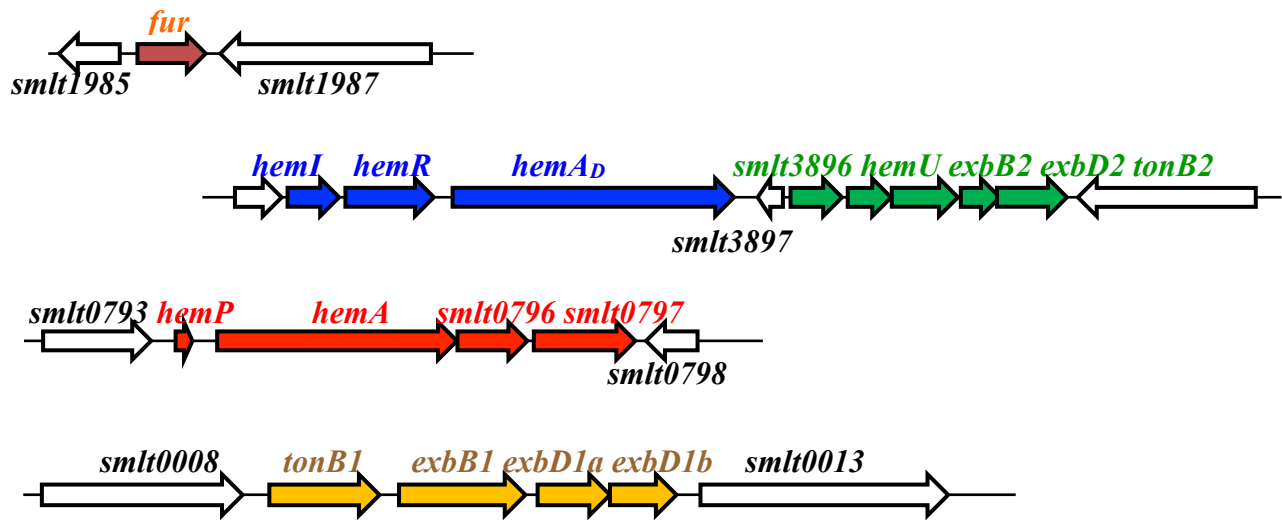


Fig. S4. Genomic organization of *fur*, *hemI-hemR-hemA_D*, *smlt3896-hemU-exbB2-exbD2-tonB2*, *hemP-hemA-smlt0796-smlt0797*, and *tonB1-exbB1-exbD1a-exbD1b* operons of *S. maltophilia*. Gene orientation is indicated by arrows. Genes belonging to the same operon are shown in the same color.

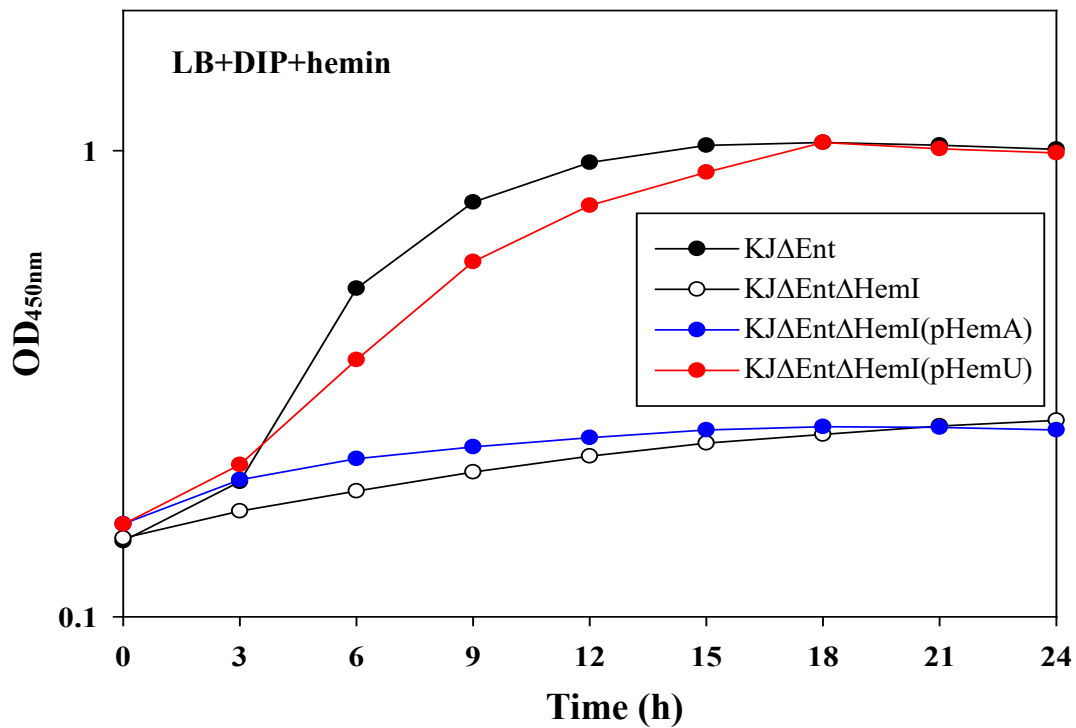
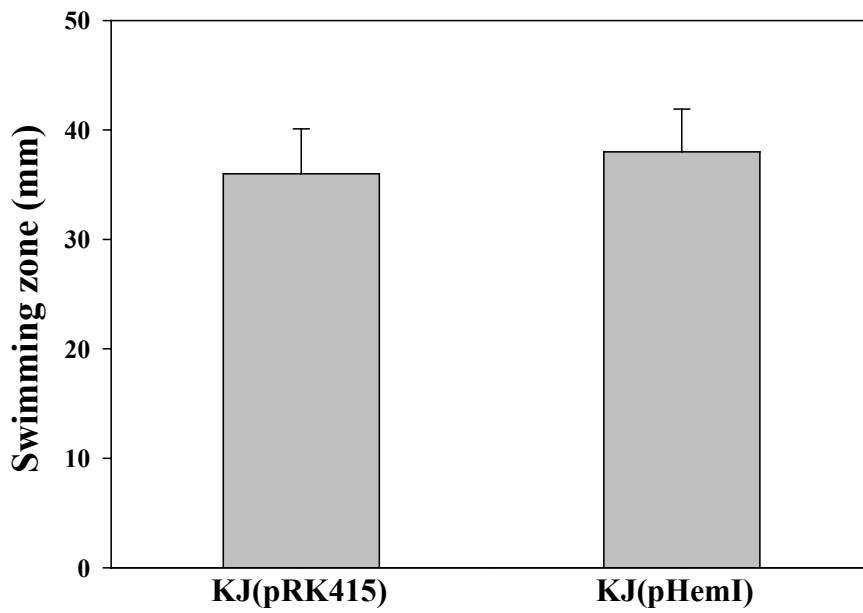


Fig. S5. Growth curve of parental strain KJΔEnt and its derived mutants grown under iron-depleted with hemin as sole iron source. An overnight bacterial culture was inoculated to LB broth supplemented with 50 μg/mL DIP and 150 μM hemin at an initial OD_{450 nm} of 0.15. Bacterial growth was monitored by recording the OD_{450 nm} for 24 h at intervals of 3 h. Graph is representative of at least three independent experiments.

(A)



(B)

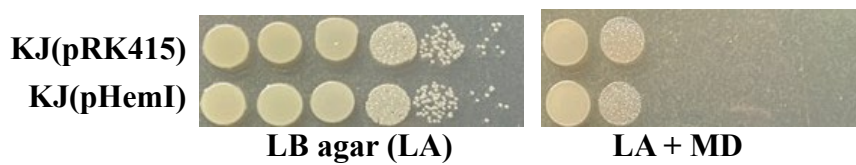


Fig. S6. Roles of HemI in swimming motility and menadione tolerance. (A) Swimming motility: overnight cultures were inoculated into swimming agar and incubated for 48 h at 37 °C. Swimming zones were recorded. Bars show the means of three independent experiments. Significance determined by Student's *t*-test. (B) Menadione tolerance: bacterial suspensions (2×10^5 CFU/ μ L) were serially diluted tenfold, and 5- μ L aliquots were spotted onto LB agar without or with 60 μ g/mL MD. Growth was assessed after 24 h at 37°C.

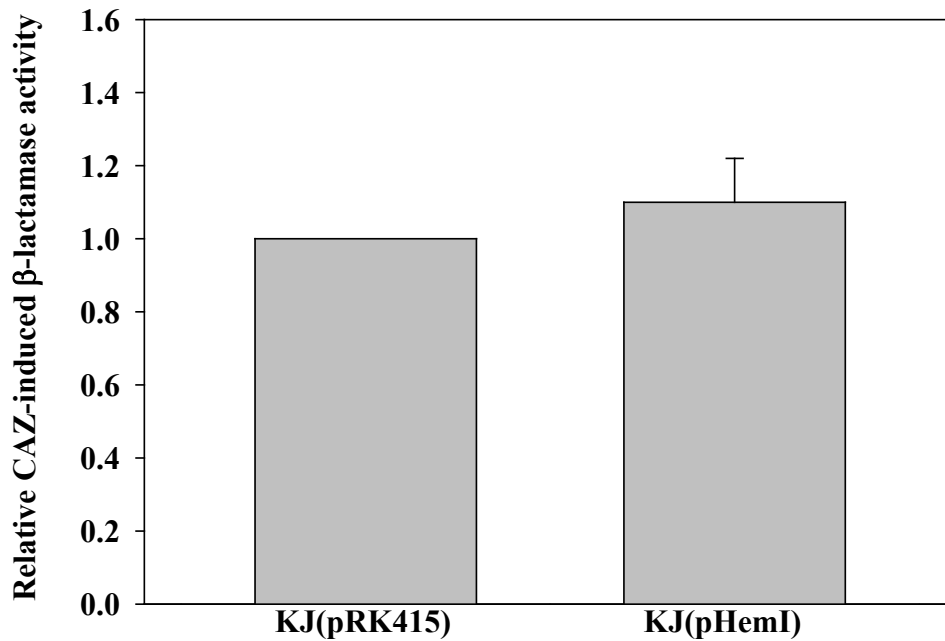


Fig. S7. Impact of HemI on CAZ-induced β -lactamase activity. Bacterial cells were grown overnight and reinoculated into 5 mL of fresh LB broth at an initial $OD_{450} = 0.15$. After 3 h, 50 $\mu\text{g/mL}$ ceftazidime was added and cultures were incubated for an additional 30 min. Intracellular β -lactamase activity was assayed using nitrocefin as the substrate. One unit (Un) of activity was defined as the amount of enzyme hydrolyzing 1 nmol of nitrocefin per min. Specific activity (Un/mg) is expressed as nmol of nitrocefin hydrolyzed per min per mg of protein. Relative β -lactamase activity was normalized to the wild-type KJ level (set as 1). Bars show the means of three independent experiments. Error bars indicate standard errors of the mean.

Table S1 Bacterial strains and plasmids used in this study

| Strain or plasmid | Genotype or properties | Reference |
|-----------------------------------|--|------------|
| <i>S. maltophilia</i> | | |
| KJ | A clinical <i>S. maltophilia</i> isolate | 1 |
| KJΔEnt | <i>S. maltophilia</i> KJ mutant of <i>entF</i> gene; nucleotides 52 to 3552 of <i>entF</i> gene deleted | 2 |
| KJΔEntΔ3897 | <i>S. maltophilia</i> KJ mutant of <i>entF</i> and <i>smlt3897</i> genes; nucleotides 52 to 3552 of <i>entF</i> gene deleted, nucleotides 43 to 174 of <i>smlt3897</i> gene deleted | This study |
| KJΔEntΔHemI | <i>S. maltophilia</i> KJ mutant of <i>entF</i> and <i>hemI</i> genes; nucleotides 52 to 3552 of <i>entF</i> gene deleted, nucleotides 91 to 363 of <i>hemI</i> gene deleted | This study |
| KJΔEntΔHemR | <i>S. maltophilia</i> KJ mutant of <i>entF</i> and <i>hemR</i> genes; nucleotides 52 to 3552 of <i>entF</i> gene deleted, nucleotides 283 to 918 of <i>hemR</i> gene deleted | This study |
| KJΔEntΔHemA _D | <i>S. maltophilia</i> KJ mutant of <i>entF</i> and <i>hemA_D</i> genes; nucleotides 52 to 3553 of <i>entF</i> gene deleted, nucleotides 460 to 2418 of <i>smlt3898</i> gene deleted | This study |
| KJΔEntΔHemI ΔHemR | <i>S. maltophilia</i> KJ mutant of <i>entF</i> , <i>hemI</i> , and <i>hemR</i> genes; nucleotides 52 to 3552 of <i>entF</i> gene deleted, nucleotides 91 to 363 of <i>hemI</i> gene deleted, nucleotides 283 to 918 of <i>hemR</i> gene deleted | This study |
| KJΔEntΔHemR ΔHemA _D | <i>S. maltophilia</i> KJ mutant of <i>entF</i> , <i>hemR</i> , and <i>hemA_D</i> genes; nucleotides 52 to 3552 of <i>entF</i> gene deleted, nucleotides 283 to 918 of <i>hemR</i> gene deleted, nucleotides 460 to 2418 of <i>smlt3898</i> gene deleted | This study |
| KJΔEntΔHemIRA _D | <i>S. maltophilia</i> KJ mutant of <i>entF</i> , <i>hemI</i> , <i>hemR</i> , and <i>hemA_D</i> genes; nucleotides 52 to 2552 of <i>entF</i> gene deleted, nucleotides 91 to 363 of <i>hemI</i> gene deleted, nucleotides 283 to 918 of <i>hemR</i> gene deleted, nucleotides 460 to 2418 of <i>smlt3898</i> gene deleted | This study |
| KJΔFur | <i>S. maltophilia</i> KJ mutant of <i>fur</i> gene; nucleotides 19 to 348 of <i>fur</i> gene deleted | 2 |
| KJΔHemP | <i>S. maltophilia</i> KJ mutant of <i>hemP</i> gene; nucleotides 31 to 171 of <i>hemP</i> gene deleted | 3 |

| | | |
|------------------------|--|------------|
| KJΔHemI | <i>S. maltophilia</i> KJ mutant of <i>hemI</i> gene; nucleotides 91 to 363 of <i>hemI</i> gene deleted | This study |
| KJΔFurΔHemI | <i>S. maltophilia</i> KJ mutant of <i>fur</i> and <i>hemI</i> genes; nucleotides 19 to 348 of <i>fur</i> gene deleted, nucleotides 91 to 363 of <i>hemI</i> gene deleted | This study |
| KJΔFurΔHemR | <i>S. maltophilia</i> KJ mutant of <i>fur</i> and <i>hemR</i> genes; nucleotides 19 to 348 of <i>fur</i> gene deleted, nucleotides 283 to 918 of <i>hemR</i> gene deleted | This study |
| KJΔFurΔHemI ΔHemR | <i>S. maltophilia</i> KJ mutant of <i>fur</i> , <i>hemI</i> , and <i>hemR</i> genes; nucleotides 19 to 348 of <i>fur</i> gene deleted, nucleotides 91 to 363 of <i>hemI</i> gene deleted, nucleotides 283 to 918 of <i>hemR</i> gene deleted | This study |
| <i>E. coli</i> | | |
| DH5α | F- φ80d/ <i>acZΔM15 Δ(lacZYA-argF)U169 deoR</i> <i>recA1 endA1 hsdR17 (r_k⁻ m_k⁺) phoA supE44λ</i> <i>thi-1 gyrA96 relA1</i> | Invitrogen |
| S17-1 | λ <i>pir</i> ⁺ mating strain | 4 |
| Plasmids | | |
| pEX18Tc | <i>sacB oriT</i> , Tc ^r | 5 |
| pRK415 | Mobilizable broad-host-range plasmid cloning vector, RK2 origin; Tc ^r | 6 |
| pΔHemI | pEX18Tc with a <i>hemI</i> gene whose nucleotides 91 to 363 were deleted, Tc ^r | This study |
| pΔHemR | pEX18Tc with a <i>hemR</i> gene whose nucleotides 283 to 918 were deleted, Tc ^r | This study |
| pΔHemA _D | pEX18Tc with a <i>smlt3898</i> gene whose nucleotides 460 to 2418 were deleted; Tc ^r | This study |
| pHemA | pRK415 with an intact <i>hemA</i> gene and ; Tc ^r | 3 |
| pHemU | pRK415 with an intact <i>hemU</i> gene; Tc ^r | 7 |
| pHemI | pRK415 with an intact <i>hemI</i> gene; Tc ^r | This study |
| pHemP _{xyIE} | pRK415 with a <i>P_{hemP}::xyIE</i> transcriptional fusion construct; Tc ^r | 3 |
| p3896 _{xyIE} | pRK415 with a <i>P_{smlt3896}::xyIE</i> transcriptional fusion construct; Tc ^r | 7 |
| pHemI _{xyIE} | pRK415 with a <i>P_{hemI}::xyIE</i> transcriptional fusion construct; Tc ^r | This study |
| pTonB1 _{xyIE} | pRK415 with a <i>P_{tonB1}::xyIE</i> transcriptional fusion construct; Tc ^r | This study |

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Table S2 Primers used in this study

| primer | Sequence (5'→3') | Amplified region | Purpose |
|--|---|--|-------------------------------------|
| HemIN-F HemIN-R | CTGAGCTCATTGCGCCAGTGACC TCGGTACCCAGGATGCGTTGCAC | 285 bps upstream of <i>hemI</i> and nucleotides 1-90 of <i>hemI</i> | pΔHemI construction |
| HemIC-F HemIC-R | CCAGGTACCGGCTCAACCGCCT GTCTCTAGAGGCCAGCCACG | nucleotides 364-501 of <i>hemI</i> and 194 bps downstream of <i>hemI</i> | |
| HemR-F HemR-R | GCAAAGCTTGACCCAGGCCGAGAT CGGTCTAGAGTGGCCGGATACACC | 195 bps upstream of <i>hemR</i> , <i>hemR</i> , and 432 bp downstream of <i>hemR</i> | pΔHemR construction |
| HemA _D -F HemA _D -R | GCCAAGCTTGGATGATGTTCCCGAT CACGAATTCGCGACCGATCAGTACT | 153 bps upstream of <i>smlt3898</i> , <i>smlt3898</i> , and 17 bp downstream of <i>smlt3898</i> | pΔHemA _D construction |
| 3897-F 3897-R | GCGTCTAGAGCAGGGTTGGCATAACA TCAGGAATTCGACAAAGGACTGGAA | 307 bps upstream of <i>smlt3897</i> , <i>smlt3897</i> , and 377 bp downstream of <i>smlt3897</i> | pΔ3897 construction |
| HemI-F HemI-R | TGCAAGCTTTGATGCGAACGTGACT ACCGAATTCACGGTCCAGTGATCAA | 84 bps upstream of <i>hemI</i> , <i>hemI</i> , and 61 bp downstream of <i>hemI</i> | pHemI construction |
| HemIN-F HemIN-R | CTGAGCTCATTGCGCCAGTGACC TCGGTACCCAGGATGCGTTGCAC | 285 bps upstream of <i>hemI</i> and nucleotides 1-89 of <i>hemI</i> | pHemI _{xyIE} construction |
| TonB1N-F TonB1N-R | CAGGTACCAGCCGGACTACCA CTTCTAGAGGGTTGTTTCGTA | 396 bps upstream of <i>tonB1</i> and nucleotides 1-48 of <i>tonB1</i> | pTonB1 _{xyIE} construction |
| HemAQ93-F HemAQ93-R | CCTGCTCAGCAAACCTGGTCT AGCACATTGGTATCGGTGGT | nucleotides 705 to 797 of <i>hemA</i> gene | qRT-PCR |
| HemUQ100-F HemUQ100-R | ATGCGCTGGCTCTGGTTC CCAGGATCAGGAAGATGGTG | nucleotides 1 to 100 of <i>hemU</i> gene | qRT-PCR |
| HemIQ110-F HemIQ110-R | GACAATCCGCGTGCTTACC GCTCCCCACAGGTAGTGGT | nucleotides 160 to 269 of <i>hemI</i> gene | qRT-PCR |
| TonB1Q100-F TonB1Q100-R | CGGTACGAACAACCCGATGA TGAGGAGCATCATGAAGGCG | nucleotides 25 to 124 of <i>tonB1</i> gene | qRT-PCR |
| 16S rDNA-F 16S rDNA-R | GACCTTGCGCGATTGAATG CGGATCGTCGCCTTGGT | nucleotides 211 to 286 of 16S rDNA | qRT-PCR |