Table S1. Sequences for phylogenetic analysis.

Names	Accession	Description	Organisms	Length [aa]	Taxonomy
AF101058 - AAD16440.1	AAD16440.1	ethylene-forming enzyme	*Pseudomonas syringae pv. Phaseolicola*	350	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
MBW4490662	MBW4490662.1	MAG: hypothetical protein KME12_23050	*Trichocoleus desertorum* ATA4-8-CV12	339	Bacteria; Cyanobacteria; Synechococcales; Trichocoleusaceae; *Trichocoleus*
MBW4538205	MBW4538205.1	MAG: hypothetical protein KME43_03555	*Myxacorys chilensis* ATA2-1-KO14	352	Bacteria; Cyanobacteria; Pseudanabaenales; Leptolyngbyaceae; *Myxacorys*; *Myxacorys chilensis*
RAM52958	RAM52958.1	MAG: MFS transporter	*Hapalosiphonaceae cyanobacterium* JJU2	360	Bacteria; Cyanobacteria; Nostocales; Hapalosiphonaceae
RCJ18531	RCJ18531.1	MFS transporter	Nostoc sp. ATCC 43529	361	Bacteria; Cyanobacteria; Nostocales; Nostocaceae; *Nostoc*
RML49514	RML49514.1	2OG-Fe oxygenase	*Pseudomonas syringae pv. pisi*	344	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*; *Pseudomonas syringae*
TMH32900	TMH32900.1	MAG: isopenicillin N synthase family oxygenase	*Betaproteobacteria bacterium*	352	Bacteria; Proteobacteria; Betaproteobacteria
WP_014618742	WP_014618742.1	isopenicillin N synthase family oxygenase	*Ralstonia solanacearum*	347	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; *Ralstonia*
WP_015351455	WP_015351455.1	isopenicillin N synthase family oxygenase	*Myxococcus stipitatus*	360	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus*
WP_019362686	WP_019362686.1	isopenicillin N synthase family oxygenase	*Pseudomonas fuscovaginae*	350	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
WP_057426137	WP_057426137.1	isopenicillin N synthase family oxygenase	*Pseudomonas syringae*	345	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
WP_063343083	WP_063343083.1	MULTISPECIES: isopenicillin N synthase family oxygenase	*Pseudomonas*	353	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae

WP_07178 0333	WP_0717803 33.1	isopenicillin N synthase family oxygenase	*Fischerella sp.* PCC 9431	385	Bacteria; Cyanobacteria; Nostocales; Hapalosiphonaceae; *Fischerella*
WP_07495 0276	WP_0749502 76.1	isopenicillin N synthase family oxygenase	*Myxococcus fulvus*	360	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus*
WP_09656 2523	WP_0965625 23.1	isopenicillin N synthase family oxygenase	*Scytonema sp.* NIES-4073	359	Bacteria; Cyanobacteria; Nostocales; Scytonemataceae; *Scytonema*; unclassified *Scytonema*
WP_11666 5714	WP_1166657 14.1	isopenicillin N synthase family oxygenase	*Pseudomonas sp.* GL93	346	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
WP_12349 6614	WP_1234966 14.1	isopenicillin N synthase family oxygenase	*Pseudomonas frederiksbergensis*	344	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
WP_13111 8892	WP_1311188 92.1	isopenicillin N synthase family oxygenase	*Mastigocladus laminosus*	360	Bacteria; Cyanobacteria; Nostocales; Hapalosiphonaceae; *Mastigocladus*
WP_14164 1058	WP_1416410 58.1	isopenicillin N synthase family oxygenase	*Myxococcus*	362	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus*
WP_16386 1341	WP_1638613 41.1	isopenicillin N synthase family oxygenase	*Myxococcus eversor*	360	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus*
WP_16398 9172	WP_1639891 72.1	isopenicillin N synthase family oxygenase	*Pyxidicoccus caerfyrddinensis*	358	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Pyxidicoccus*
WP_16934 3509	WP_1693435 09.1	isopenicillin N synthase family oxygenase	*Pyxidicoccus fallax*	356	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Pyxidicoccus*
WP_17447 5597	WP_1744755 97.1	isopenicillin N synthase family oxygenase	*Myxococcus sp.* CA033	360	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus*; unclassified *Myxococcus*
WP_17643 4236	WP_1764342 36.1	isopenicillin N synthase family oxygenase	*Myxococcus sp.* AM011	360	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus*; unclassified *Myxococcus*
WP_18108 0955	WP_1810809 55.1	isopenicillin N synthase family oxygenase	*Pseudomonas lurida*	351	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*

WP_181286820	WP_181286820.1	isopenicillin N synthase family oxygenase	*Pseudomonas brassicacearum*	346	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
WP_191133867	WP_191133867.1	isopenicillin N synthase family oxygenase	*Phormidium tenue*	352	Bacteria; Cyanobacteria; Oscillatoriophycideae; Oscillatoriales; Oscillatoriaceae; *Phormidium*
WP_193874723	WP_193874723.1	isopenicillin N synthase family oxygenase	*Oculatella sp.* LEGE 06141	356	Bacteria; Cyanobacteria; Pseudanabaenales; Oculatellaceae; *Oculatella*; unclassified *Oculatella*
WP_206715722	WP_206715722.1	isopenicillin N synthase family oxygenase	*Myxococcus* sp. SCHIC003	360	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus;* unclassified *Myxococcus*
WP_216670419	WP_216670419.1	hypothetical protein	*Microcoleus asticus*	369	Bacteria; Cyanobacteria; Oscillatoriophycideae; Oscillatoriales; Microcoleaceae; *Microcoleus*
WP_217855079	WP_217855079.1	MFS transporter	*Pseudomonas asgharzadehiana*	339	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
WP_223018911	WP_223018911.1	MFS transporter	*Pseudomonas sp*. P867	346	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
WP_223745345	WP_223745345.1	MFS transporter	*Myxococcus sp.* XM-1-1-1	360	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus*; unclassified *Myxococcus*
WP_223781934	WP_223781934.1	MFS transporter	*Myxococcus sp*. AS-1-15	352	Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae; Myxococcaceae; *Myxococcus*; unclassified *Myxococcus*
WP_229496330	WP_229496330.1	hypothetical protein	*Nostoc mirabile*	360	Bacteria; Cyanobacteria; Nostocales; Nostocaceae; *Nostoc*

Table S2. Parameters of the five constructed models based on 6VP4.1.A.

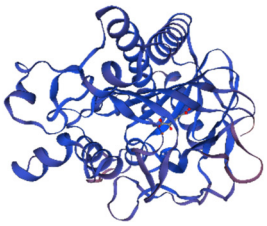
Proteins	Oligo-State	Seq Identity	GMQE	QMEANDisCo Global	Ligands
Efe_MS	Monomer	63.29%	0.87	0.86 ± 0.05	1xAKG, 1xFE
Efe_RS	Monomer	61.40%	0.87	0.84 ± 0.05	1xAKG, 1xFE
Efe_MA	Monomer	67.74%	0.85	0.87 ± 0.05	1xARG, 1xAKG, 1xFE
Efe_NS	Monomer	67.25%	0.87	0.87 ± 0.05	1xAKG, 1xFE
Efe_SS	Monomer	68.10%	0.88	0.87 ± 0.05	1xARG, 1xAKG, 1xFE

* 1 x AKG: α-ketoglutaric acid ligand binding sites; 1 x ARG: arginine ligand binding sites; 1 x FE: FE (III) ion ligand binding sites.

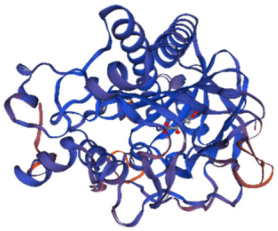
*GMQE (Global Model Quality Estimate) and QMEANDisCo global (QMEAN: Qualitative Model Energy Analysis) give an overall model quality measurement between 0 and 1, with higher numbers indicating higher l expected quality. GMQE is coverage dependent, i.e., a model covering only half of the target sequence is unlikely to get a score above 0.5 QMEANDisCo on the other hand evaluates the model 'as is' without explicit coverage dependency.

(a)

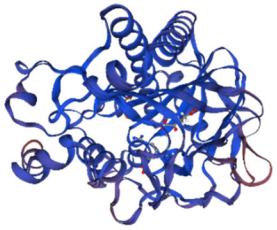
Efe_MS



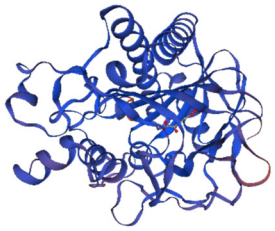
Efe_RS



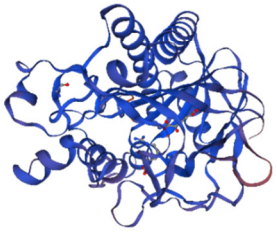
Efe_MA

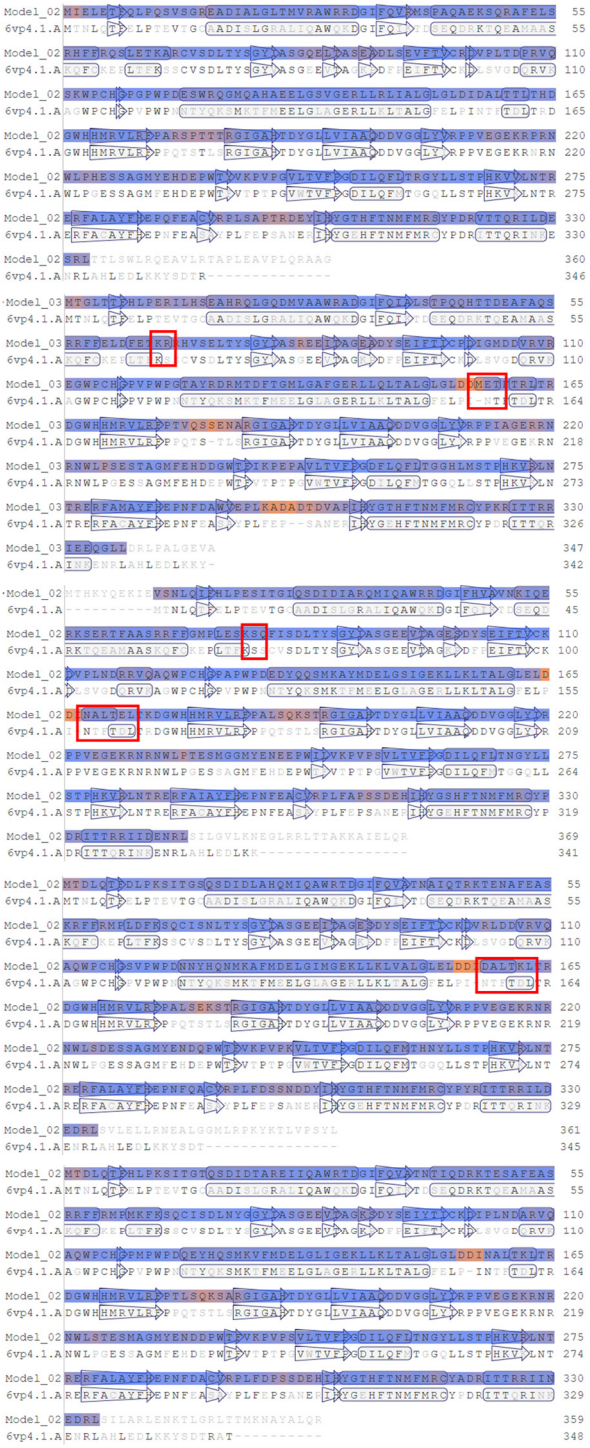


Efe_NS

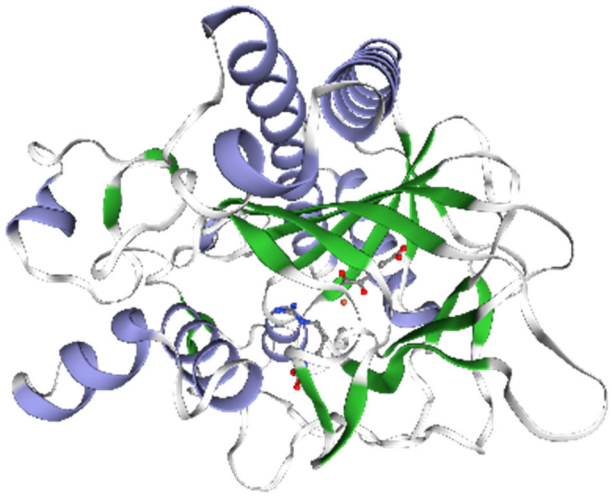


Efe_SS



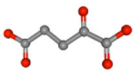


(b)

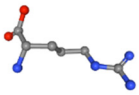


Efe_PS-AAD16440.1（6vp4.1.A）

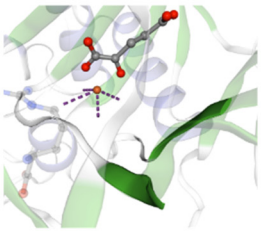
FE(III)ION



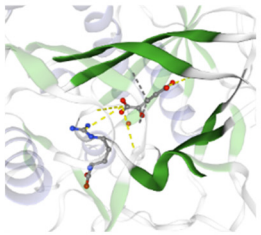
2-OXOGLUTARIC ACID



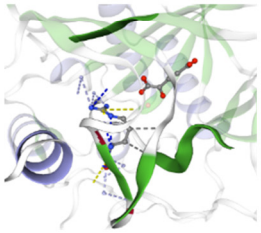
ARGININE



FE site



AKG site



ARG site

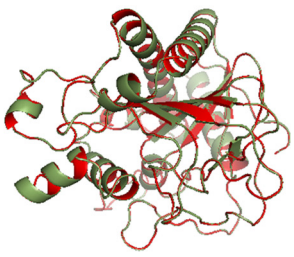
All the six

Efe_MS RMSD=0.057 (322 to 322 atoms)

Efe_RS RMSD=0.056 (297 to 297 atoms)

Efe_MA RMSD=0.055 (312 to 312 atoms)

Efe_NS RMSD=0.054 (314 to 314 atoms)



Efe_SS RMSD=0.058 (318 to 318 atoms)

Figure S1. Model of Efes, 6VP4.1.A and its active sites. (**a**) Efe models constructed by SWISS MODEL and structure aligning to 6VP4.1.A. They are shown by confidence level: blue—red: high confidence—low confidence. α-helix: labeled with the arrow; β-helix: labeled with square. Little change shows in α-helix or β-helix and most sites are in high confidence. Changes are marked by red box. (**b**) Model of 6VP4.1.A and its active sites (α-helix: purple; β-helix: green; active sites are darker in the right figures). (c) Structure alignment by PyMOL between five Efes and 6vp4.1.A (in red). RMSD root-mean-square deviation, a parameter used to indicate differences in atomic positions between protein structures. RMSDs of these five aligning are all less than 0.1 (abnormal atoms were rejected during the cycle), which means these models have little difference in structures. There are slight fluctuations in atomic positions between protein structures, while the main frameworks remain the same.

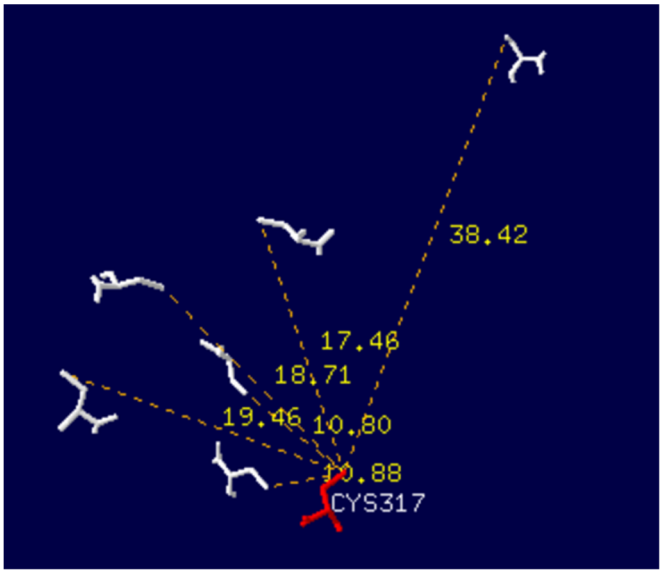


Figure S2. Analysis of disulfide bond formation of cysteine residue in the original model 6VP4.1.A.

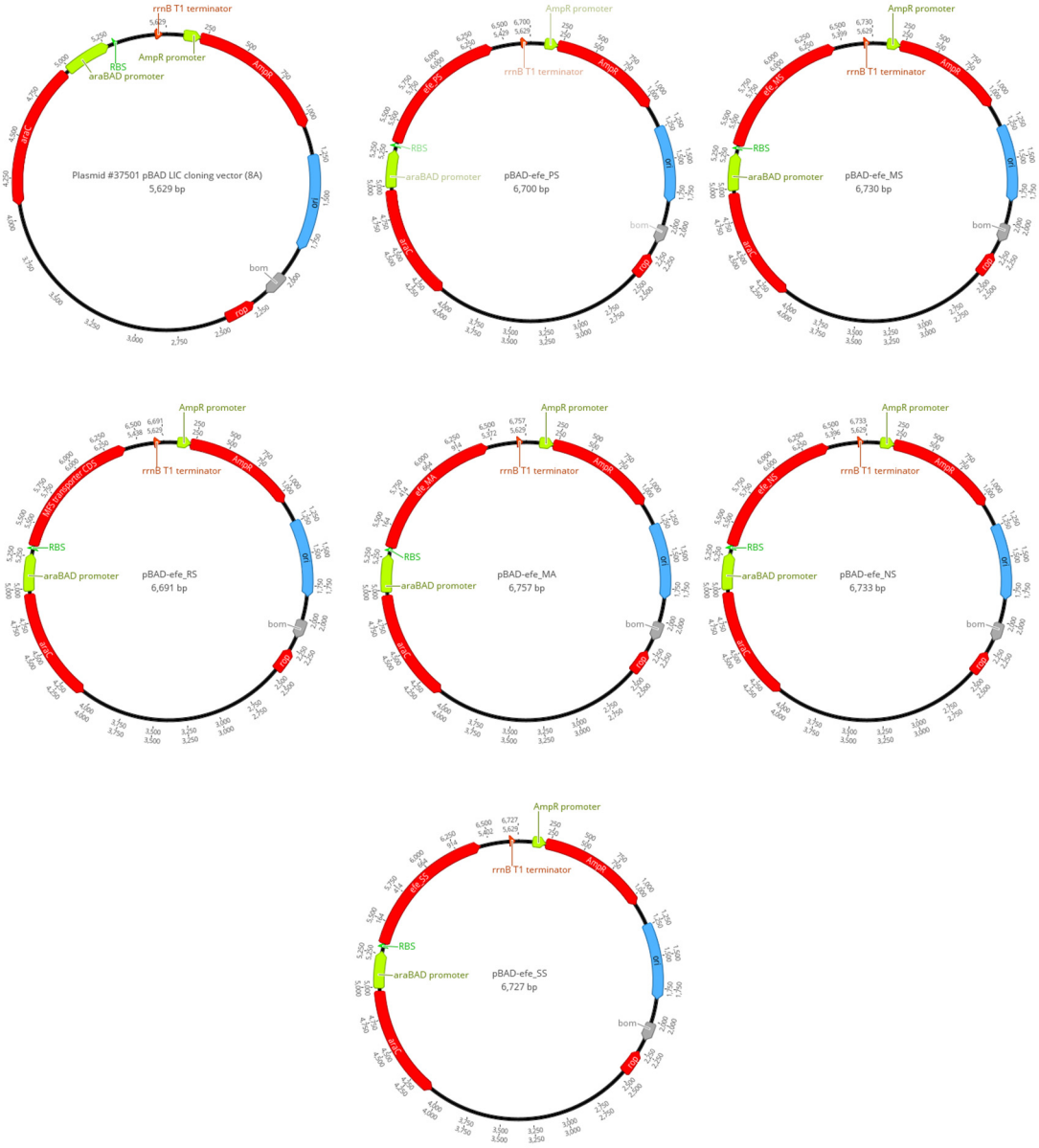


Figure S3. Plasmids profiles of pBAD_LIC_cloning vector and the six recombinant plasmids.

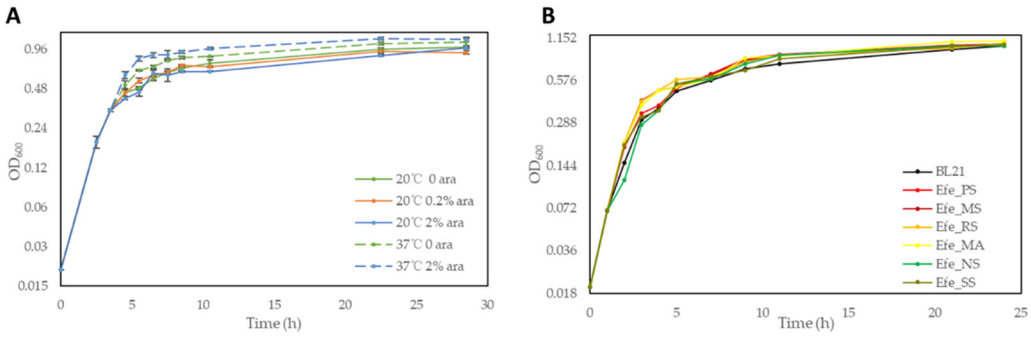


Figure S4. Growth curves of BL21_*efe*+ in LB medium with arabinose and antibiotic (ampicillin) in low temperature. (**A**). Growth in LB with arabinose concentration of 0 (green), 0.2% (orange) and 2% (blue) under 20 °C (full line) or 37 °C (dashed line); (**B**). Growth curve of the six engineering strains in LB with 2% arabinose and 50 μg/mL ampicillin: black: BL21_WT; red: Efe_PS; rose red: Efe_MS; orange: Efe_RS; yellow: Efe_MA; green: Efe_NS; brown: Efe_SS.

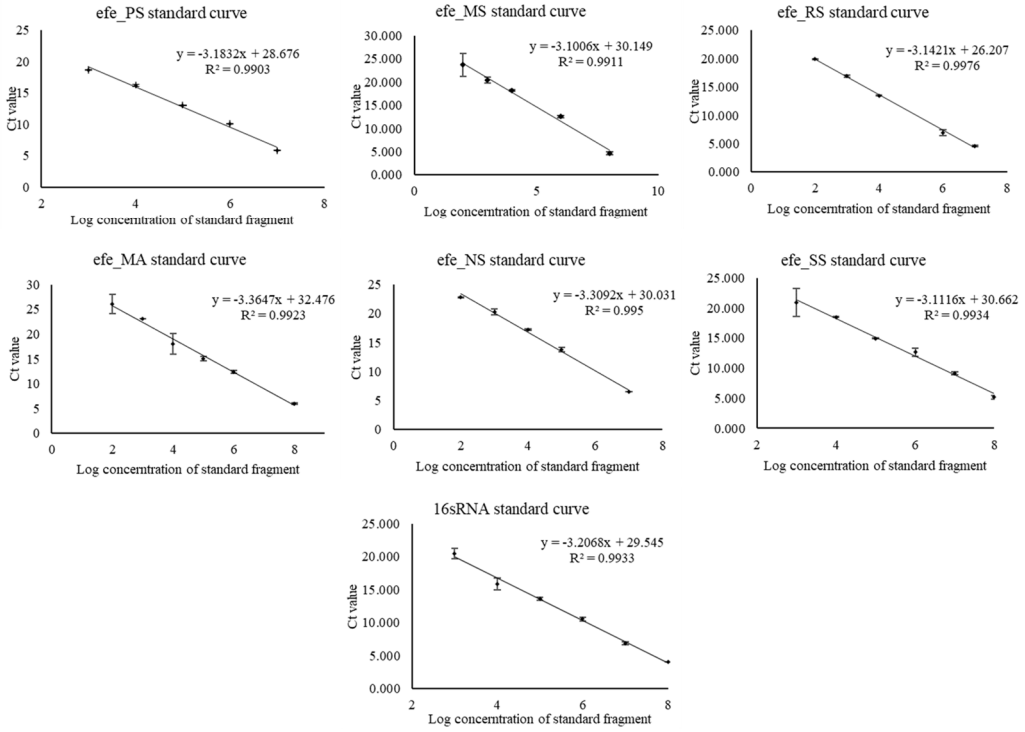


Figure S5. RT-qPCR standard curves of *efe* genes and 16sRNA gene.

Table S3. Protein MS (Q-E test) results of Efes.

Accession	Mass	Score	Matches1	Sequences2	emPAI3	Coverage
Efe_NS	39593	9040	268(233)	35(27)	49.86	71%
Efe_PS	39819	3179	160(113)	37(20)	12.90	70%
Efe_RS	41756	2542	89(70)	30(18)	12.35	68%
Efe_MS	40953	2060	129(74)	32(15)	8.50	58%
Efe_SS	39223	1406	70(33)	24(10)	1.44	47%
Efe_MA	42477	634	53(24)	21(10)	1.85	30%

[1]Matches: the total number of peptide matches, in parentheses, is the number of matches above the significance threshold；[2]Sequences: the total number of matched sequences is the number of sequences higher than the significance threshold in parentheses; [3]emPAI：$10^{(\text{Nobserved/Nobservable})-1}$

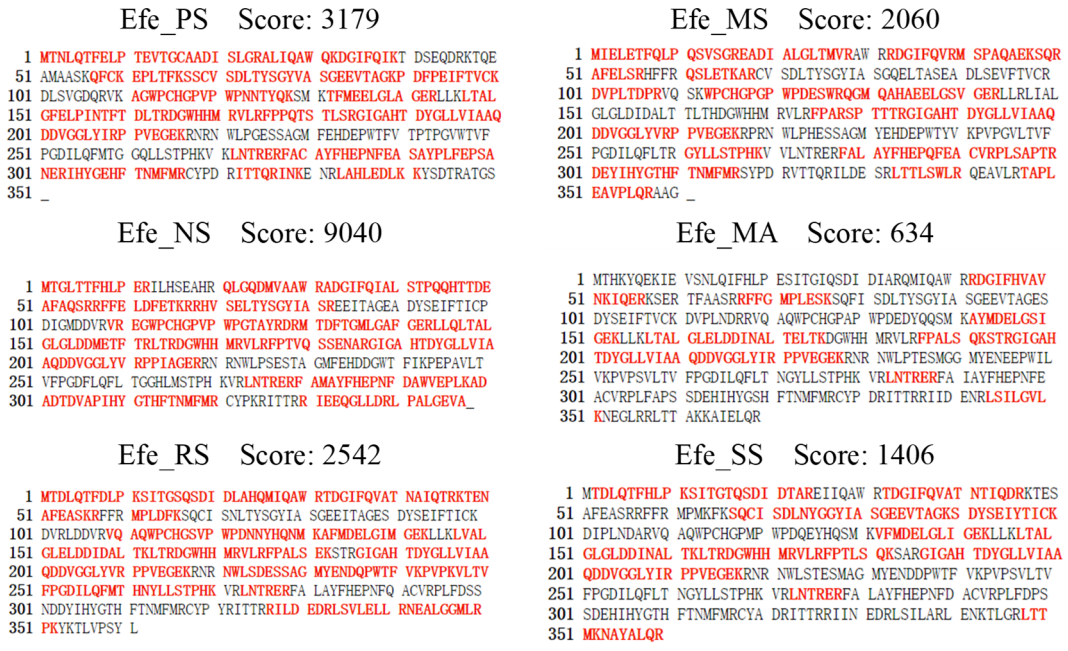


Figure S6. Protein MS sequence alignment.