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Supplementary information for:

Efficient biosynthesis of 5-aminolevulinic acid from glutamate via whole-cell biocatalyst in immobilized engineered *Escherichia coli*

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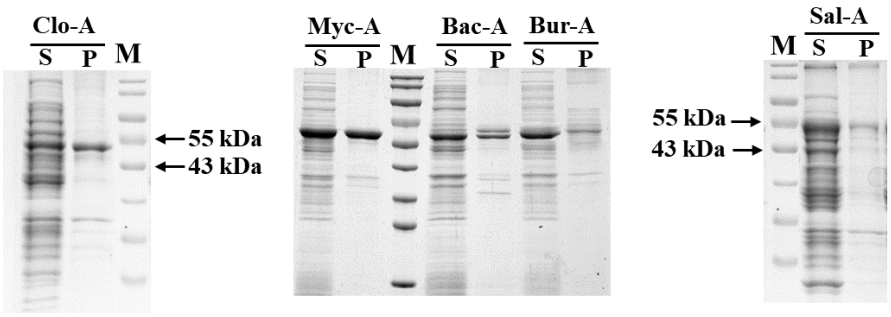

Figure S1. SDS-PAGE analysis of protein expression at 12 h and HemA is indicated by the arrow. S stands for soluble proteins, while P is the insoluble in the pellet. M is the protein marker with the corresponding molecular weight (kDa) beside the bands. The size of HemA protein from *C. beijerinckii M. diernhoferi*, *B. cereus*, *Burkholderia caryophylli* and *S. typhimurium* is 46.92 kDa, 48.23 kDa, 49.28 kDa, 47.87 kDa, and 46.12 kDa, respectively.

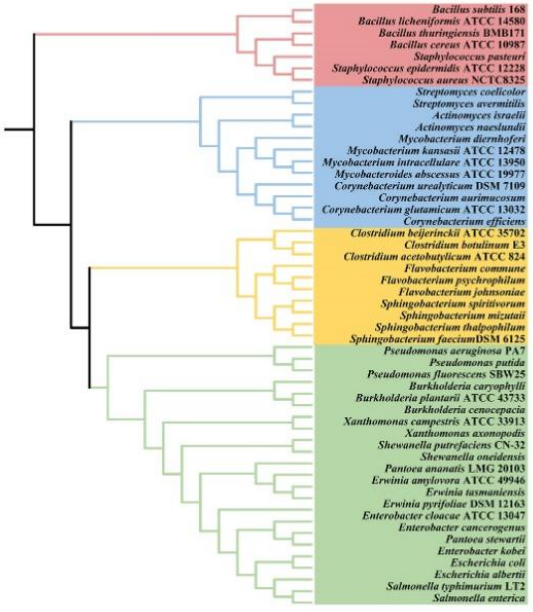

Figure S2 Phylogenetic tree based on merged sequences of GltX and HemA. The tree

was constructed based on amino acid sequences from 51 various bacterial organisms

Table S1 Plasmids used in this study

Plasmids	Relevant characteristics	Sources
pET30a	pBR322 ori, T7 promoter, Km^r, *E. coli* expression vector	This work
pET30a-*ClohemA*	pET30a containing *hemA* gene (*C. beijerinckii*)	This work
pET30a-*MychemA*	pET30a containing *hemA* gene (*M. diernhoferi*)	This work
pET30a-*BachemA*	pET30a containing *hemA* gene (*Bacillus cereus*)	This work
pET30a-*BurhemA*	pET30a containing *hemA* gene (*Burkholderia caryophylli*)	This work
pET30a-*SalhemA*	pET30a containing *hemA* gene (*S. typhimurium*)	This work
pET30a-*ClohemA*-*gltX*-$tRNA^{Glu}$	pET30a containing *hemA, gltX* and $tRNA^{Glu}$ gene (*C. beijerinckii*)	This work
pET30a-*MychemA*-*gltX*-$tRNA^{Glu}$	pET30a containing *hemA, gltX* and $tRNA^{Glu}$ gene (*M. diernhoferi*)	This work
pET30a-*BachemA*-*gltX*-$tRNA^{Glu}$	pET30a containing *hemA, gltX* and $tRNA^{Glu}$ gene (*Bacillus cereus*)	This work
pET30a-*BurhemA*-*gltX*-$tRNA^{Glu}$	pET30a containing *hemA, gltX* and $tRNA^{Glu}$ gene (*Burkholderia caryophylli*)	This work
pET30a-*SalhemA*-*gltX*-$tRNA^{Glu}$	pET30a containing *hemA, gltX* and $tRNA^{Glu}$ gene (*S. typhimurium*)	This work
pET30a-*BurhemA*-$tRNA^{Glu}$-*SalhemL*	pET30a containing *hemA,* $tRNA^{Glu}$ (*Burkholderia caryophylli*) and *gltX* gene (*S. typhimurium*)	This work
pET30a-*ClohemA*-*gltX*-*hemL*-$tRNA^{Glu}$	pET30a containing *hemA, gltX*, *hemL* and $tRNA^{Glu}$ gene (*C. beijerinckii*)	This work
pET30a-*MychemA*-*gltX*-*hemL*-$tRNA^{Glu}$	pET30a containing *hemA, gltX*, *hemL* and $tRNA^{Glu}$ gene (*M. diernhoferi*)	This work
pET30a-*BachemA*-*gltX*-*hemL*-$tRNA^{Glu}$	pET30a containing *hemA, gltX*, *hemL* and $tRNA^{Glu}$ gene (*Bacillus cereus*)	This work
pET30a-*BurhemA*-*gltX*-*hemL*-$tRNA^{Glu}$	pET30a containing *hemA, gltX*, *hemL* and $tRNA^{Glu}$ gene (*Burkholderia caryophylli*)	This work
pET30a-*SalhemA*-*gltX*-*hemL*-$tRNA^{Glu}$	pET30a containing *hemA, gltX*, *hemL* and $tRNA^{Glu}$ gene (*S. typhimurium*)	This work

pAtSSL0 (pET30a-*BurhemA*-tRNA^{Glu}-*SalgltX-hemL*)	pET30a containing *hemA* (*Burkholderia caryophylli*) *gltX* and *hemL* (*S. typhimurium*) located tandemly in one operon and tRNA^{Glu} (*Burkholderia caryophylli*) located in one operon	This work
pAtSSL1	pET30a containing *hemA* (*Burkholderia caryophylli*) and *gltX* (*S. typhimurium*) located tandemly in one operon, *hemL* (*S. typhimurium*) and tRNA^{Glu} (*Burkholderia caryophylli*) with two separated operons	This work
pAtSSL2	pET30a containing *gltX* and *hemL* (*S. typhimurium*) located tandemly in one operon, *hemA* and tRNA^{Glu} (*Burkholderia caryophylli*) with two separated operons	This work
pAtSSL3	pET30a containing *hemA* (*Burkholderia caryophylli*) and *hemL* (*S. typhimurium*) located tandemly in one operon, *gltX* (*S. typhimurium*) and tRNA^{Glu} (*Burkholderia caryophylli*) with two separated operons	This work
pAtSSL4	pET30a containing *hemA* (*Burkholderia caryophylli*) *gltX* and *hemL* (*S. typhimurium*) located tandemly in one operon and tRNA^{Glu} (*Burkholderia caryophylli*) with four separated operons	This work

Table S2. Primers used in this study

Primer	Sequence (5'-3')	Relevance
CloA-F	ccatggctgatatcggatccGTGATAGGCTTAATAGGCATAAGAA	
CloA-R	cggagctcgaattcggaCTATTCCTTCGTCATAAATATTTTC	
MycA-F	ccatggctgatatcggatccGTGAGCGTGCTGCTATTCG	pET30a-*ClohemA*,
MycA-R	cggagctcgaattcggaCTACAACTCAGGCTTGTCGTTC	pET30a-*MychemA*,
BacA-F	ccatggctgatatcggatccGTGCATATTCTTGTTGTTAGTGTAA	pET30a-*BachemA*,
BacA-R	tcgagtgcggccgcaagcttTTATAAAGATGGAACGGATGGTGT	pET30a-*BurhemA*,
BurA-F	ccatggctgatatcggatccATGCAACTGCTTACGATCGGAAT	pET30a-*SalhemA*
BurA-R	cggagctcgaattcggaCTAATGATCGGAGGGATCGGA	construction
SalA-F	ccatggctgatatcggatccATGACCCTTTTAGCGCTCGGTA	
SalA-R	tcgagtgcggccgcaagcttCTACTCCAGCCCGAGGCT	
CloS-F	atccgaattcgagctcGAAGGAGATATACATATGAGTTTTGAAAAATTAGCAGATA	pET30a-*ClohemA*-*gltX*-tRNA^{Glu},
CloS-R	tggtggtggtggtgctcgagTTATATATTTAAAAATTTATCAAAT	pET30a-*MychemA*-
MycS-F	cgagctccgtcgacaagcttGAAGGAGATATACATGTCGATTCCGTCCGTCC	*gltX*-tRNA^{Glu},
MycS-R	tcgagtgcggccgcaagcTCACACCCCACCCCGTAC	pET30a-*BachemA*-
BacS-F	tttataaaagcttgcGAAGGAGATATACATATGGAAGGTGTACCAATTATGGAAA	*gltX*-tRNA^{Glu},
		pET30a-*BurhemA*-
BacS-R	gtggtgctcgagtgcTTAACCAATTACTTTTTGAATACGA	*gltX*-tRNA^{Glu} and
BurS-F	ctccgatcattagcgGAAGGAGATATACATGTCCAACGTCCGTACCC	pET30a-*SalhemA*-
BurS-R	tgtcgacggagctcgaattcTCAGCCGGCGAGCCCTTT	*gltX*-tRNA^{Glu}
SalS-F	ggagtagaagcttgcGAAGGAGATATACATATGAAAATCAAAACTCGCTTCGCG	construction

SalS-R	gtggtgctcgagtgcTTACTGCTGACTTTCACGCTCG	
SalS-F1	ctccgatcattagcgGAAGGAGATATACATGAAAATCAAAACTCGCTTCGCG	
SalS-R1	tgtcgacggagctcgaattcTTACTGCTGACTTTCACGCTCG	
tRNA-F	aaggaatggtgcatgTAATACGACTCACTATAGGGGAATT	
tRNA-R	ccatctccttgcatgCAAAAAACCCCTCAAGACCCGT	
CloL-F	atccgaattcgagctcGAAGGAGATATACTTGAAAAACGTTGATATATTTAAAG	pET30a-*ClohemA-gltX-hemL*-tRNA^{Glu}, pET30a-*MychemA-gltX-hemL*-tRNA^{Glu}, pET30a-*BachemA-gltX-hemL*-tRNA^{Glu}, pET30a-*BurhemA-gltX-hemL*-tRNA^{Glu}, pET30a-*SalhemA-gltX-hemL*-tRNA^{Glu} construction
CloL-R	cttgtcgacggagctTTATAAACTCATTTTATTTTCATTT	
MycL-F	ctgagttgtagtccgaattctGAAGGAGATATACATGACAAGCACGGCCGCC	
MycL-R	tgtcgacggagctcgaatTCACGGGGTGGCCTCG	
BacL-F	taattggttaagcacGAAGGAGATATACATATGAAAAAGTTTGATAAGTCGATTG	
BacL-R	tggtggtggtggtgcTTAAGCTTTTAATTTTGACATTGCA	
BurL-F	cgtcgacaagcttgcGAAGGAGATATACATGTCCAGCAACCAAGCCCTTT	
BurL-R	gtggtgctcgagtgcggccgcTCAGGCCGCGAGCGAG	
SalL-F	gtcagcagtaagcacGAAGGAGATATACATATGAGTAAGTCTGAAAATCTCTATA	
SalL-R	tggtggtggtggtgcTTACAGTTTCGCAAACACCCGA	
SalL-F1	ctccgatcattagcgGAAGGAGATATACATGAGTAAGTCTGAAAATCTCTATA	pAtSSL0 construction
SalL-R1	tgtcgacggagctcgaattcTTACAGTTTCGCAAACACCCGA	
PTSalL-F	agaaggagatatacatatgAGTAAGTCTGAAAATCTCTATA	pAtSL1, pAtSL2, pAtSL3 and pAtSL4 construction
PTSalL-R	tgtcgacggagctcgaattcTTACAGTTTCGCAAACACCCGA	
PciF	CCTTTTGCTCACATGTCGATCCCGCGAAATTAATACG	
PciR	AGTCGTATTAACACGGATATAGTTCCTCCTTTCAGC	
PciF1	tggccttttgctcacatgtCGATCCCGCGAAATTAATACG	
PciR1	ctatagtgagtcgtattaacacGGATATAGTTCCTCCTTTCAGC	
PTSalS-F	agaaggagatatacatATGAAAATCAAAACTCGCTTCGCG	
PTSalS-R	tgtcgacggagctcgaattcTTACTGCTGACTTTCACGCTCG	
PTSalSL-F	agaaggagatatacatATGAAAATCAAAACTCGCTTCGCG	
PTSalSL-R	tgtcgacggagctcgaattcTTACAGTTTCGCAAACACCCGA	