

Enhanced Phycocyanobilin Production in *Escherichia coli* by Fusion-Expression of Apo-Proteins with Signal Peptides

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Supporting Information

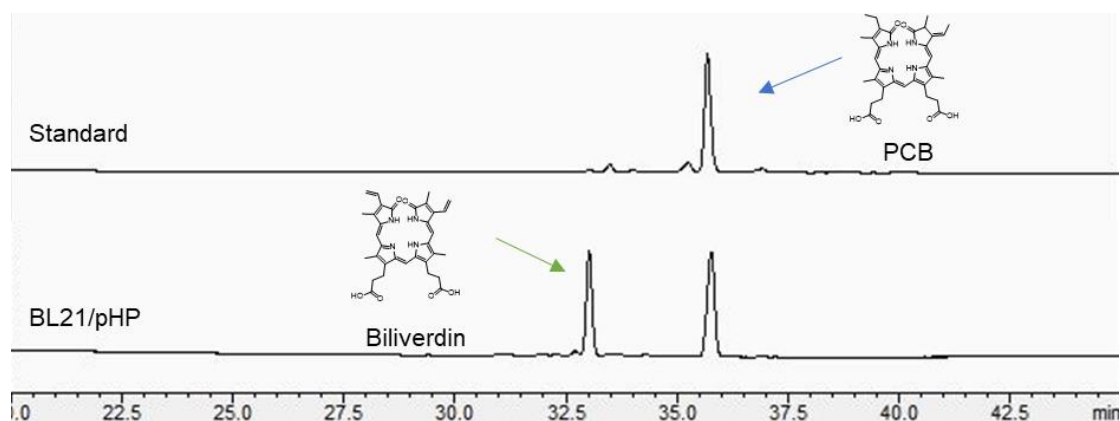


Figure S1. HPLC analysis of BL/pHP strain.

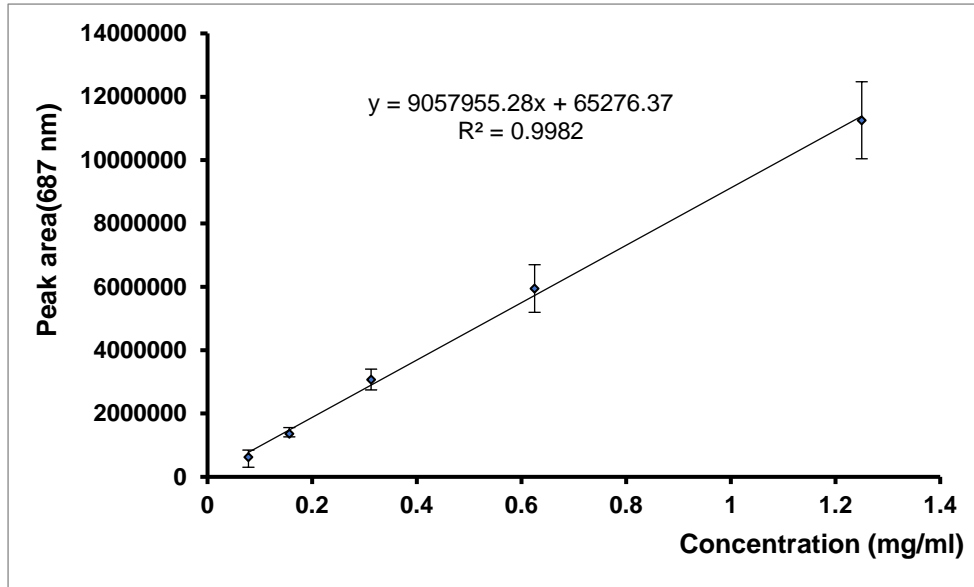


Figure S2. Standard curve for PCB using HPLC at 687 nm.

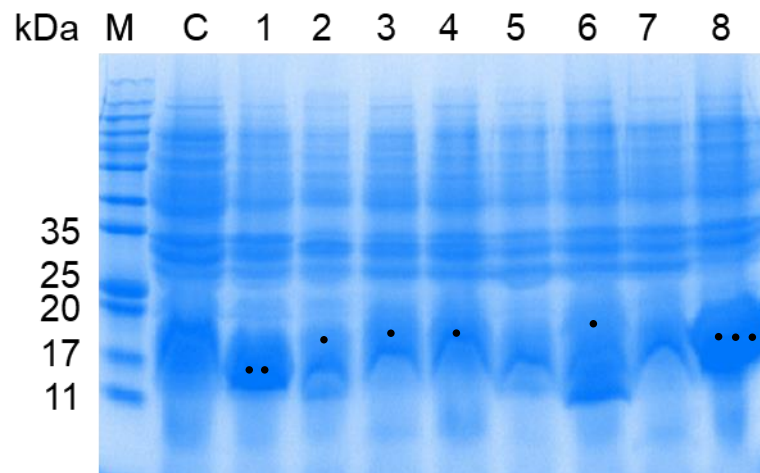


Figure S3. SDS-PAGE of expression of pET28a-sp-*cpcA*. M: Marker; C: Control, pET28a; Lanes: 1, pET28a-*cpcA*; 2, pET28a-pelB-*cpcA*; 3, pET28a-KP-SP-*cpcA*; 4, pET28a-PhoA-*cpcA*; 5, pET28a-OmpA-*cpcA*; 6, pET28a-MalE-*cpcA*; 7, pET28a-Lpp-*cpcA*; 8, pET28a-N20-*cpcA*.

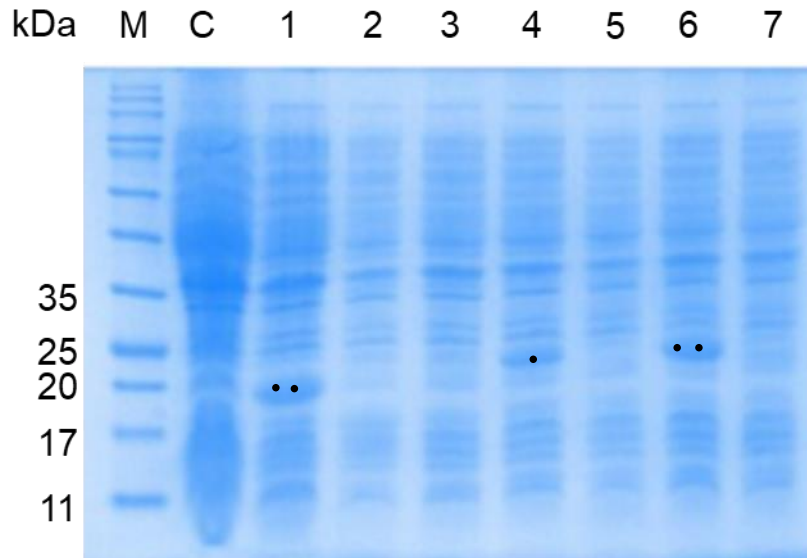


Figure S1. SDS-PAGE of expression of pET28a-sp-*cpcB*. M: Marker; C: Control, pET28a; Lanes: 1, pET28a-*cpcB*; 2, pET28a-pelB-*cpcB*; 3, pET28a-KP-SP-*cpcB*; 4, pET28a-PhoA-*cpcB*; 5, pET28a-OmpA-*cpcB*; 6, pET28a-MalE-*cpcB*; 7, pET28a-Lpp-*cpcB*.

Table S1. Recipes of the media used in this study

	LB	SOB	SOC	2xYT	TB	Superbroth
Tryptone (g/L)	10	20	20	16	12	32
Yeast extract (g/L)	5	5	5	10	24	20
NaCl (g/L)	10	0.58	0.58	5	-	5
Glycerol (ml/L)	-	-	-	-	4	-
Phosphate buffer (0.17 M KH_2PO_4 , 0.72 M K_2HPO_4) (ml/L)	-	-	-	-	100	-
KCl (g/L)	-	1.9	1.9	-	-	-
MgCl_2 (g/L)	-	0.95	0.95	-	-	-
MgSO_4 (g/L)	-	1.21	1.21	-	-	-
Glucose (g/L)	-	-	3.6	-	-	-

Table S2. Plasmids and strains used in this work

Plasmid/strain	Relevant characteristics	Sources or references
Plasmid		
pET28a(+)	Plasmid for gene expression, Kanamycin resistance	Lab stock
pET21c(+)	Plasmid for gene expression, Ampicillin resistance	Lab stock

pETDuet-1	Plasmid for gene expression, Ampicillin resistance	Lab stock
pACYCDDuet-1	Plasmid for gene expression, Chloramphenicol resistance	Lab stock
pHP	pET21c(+) containing <i>ho1</i> and <i>pcyA</i> , whose expressions are regulated by T7 promoter	This work
pDHP	pETDuet-1 containing <i>ho1</i> and <i>pcyA</i> , whose expressions are regulated by T7 promoter	This work
pAHP	pACYCDDuet-1 containing <i>ho1</i> and <i>pcyA</i> , whose expressions are regulated by T7 promoter	This work
pFEA	pET28a(+) containing <i>cpcF</i> , <i>cpcE</i> and <i>cpcA</i> , whose expressions are regulated by T7 promoter	This work
pBS	pET28a(+) containing <i>cpcB</i> and <i>cpcS</i> , whose expressions are regulated by T7 promoter	This work
pBT	pET28a(+) containing <i>cpcB</i> and <i>cpcT</i> , whose expressions are regulated by T7 promoter	This work
pET28a-sp- <i>cpcA</i>	pET28a- <i>cpcA</i> carrying N20, OmpA, pelB, LPP, PhoA, MalE, Kp-SP signal peptide, respectively.	This work
pET28a-sp- <i>cpcB</i>	pET28a- <i>cpcB</i> carrying OmpA, pelB, LPP, PhoA, MalE, Kp-SP signal peptide, respectively.	This work
pFENA	pET28a(+) containing <i>cpcF</i> , <i>cpcE</i> and N20- <i>cpcA</i> , whose expressions are regulated by T7 promoter	This work
pPBT	pET28a(+) containing <i>phoA-cpcB</i> and <i>cpcT</i> , whose expressions are regulated by T7 promoter	This work
pMBT	pET28a(+) containing <i>malE-cpcB</i> and <i>cpcT</i> , whose expressions are regulated by T7 promoter	This work
Strain		
<i>E. coli</i> DH5α	The cloning host for plasmid construction	Lab stock
<i>E. coli</i> BL21(DE3)	The expression host for protein expression	Lab stock
BL21/pHP	<i>E. coli</i> BL21(DE3) carrying pET21c- <i>ho1-pcyA</i> plasmid	This work

BL21/pFEA/pHP	<i>E. coli</i> BL21(DE3) carrying pET21c- <i>hol-pcyA</i> and pET28a- <i>cpcF-cpcE-cpcA</i> plasmids	This work
BL21/pFENA/pHP	<i>E. coli</i> BL21(DE3) carrying pET21c- <i>hol-pcyA</i> and pET28a- <i>cpcF-cpcE-N20-cpcA</i> plasmids	This work
BL21/pBS/pHP	<i>E. coli</i> BL21(DE3) carrying pET21c- <i>hol-pcyA</i> and pET28a- <i>cpcB-cpcS</i> plasmids	This work
BL21/pBT/pHP	<i>E. coli</i> BL21(DE3) carrying pET21c- <i>hol-pcyA</i> and pET28a- <i>cpcB-cpcT</i> plasmids	This work
BL21/pPBT/pHP	<i>E. coli</i> BL21(DE3) carrying pET21c- <i>hol-pcyA</i> and pET28a- <i>phoA-cpcB-cpcT</i> plasmids	This work
BL21/pMBT/pHP	<i>E. coli</i> BL21(DE3) carrying pET21c- <i>hol-pcyA</i> and pET28a- <i>malE-cpcB-cpcT</i> plasmids	This work
BL21/pFENA/pDHP	<i>E. coli</i> BL21(DE3) carrying pETDuet- <i>hol-pcyA</i> and pET28a- <i>cpcF-cpcE-N20-cpcA</i> plasmids	This work
BL21/pFENA/pAHP	<i>E. coli</i> BL21(DE3) carrying pACYCDuet- <i>hol-pcyA</i> and pET28a- <i>cpcF-cpcE-N20-cpcA</i> plasmids	This work
BL21/pFENA/pDHP/pAHP	<i>E. coli</i> BL21(DE3) carrying pETDuet- <i>hol-pcyA</i> , pACYCDuet- <i>hol-pcyA</i> and pET28a- <i>cpcF-cpcE-N20-cpcA</i> plasmids	This work

Table S3. Primers used in this study

Primer	Sequence
OL-cpcA-F	atgaaaaccctttaactgaagccgtttc
OL-cpcA-R	acgcgtcgaccgtacgactagtctagctcagag
OL-ompA-F	ggaattccatatgaagaagaccgcaattgc
OL-OmcpA-R	gaaacggcttcagttaaaggggtttcatggcctgtgcaacgggtggc
OL-pelB-F	ggaattccatatgaaatatctgctgccgaccg
OL-PecpcA-R	cggcttcagttaaaggggtttcattgccatggccggctggg
OL-Lpp-F	ggaattccatatgaaagcaaccaaactggtgc
OL-LpcpcA-R	gaaacggcttcagttaaaggggtttcataccggccagcagggtg
OL-PhoA-F	ggaattccatatgaaacagagcaccattgcac
OL-PhcpcA-R	cggcttcagttaaaggggtttcatggcttggtcaccgggg
OL-malE-F	ggaattccatatgaaaattaagaccggtgcacg
OL-MacpcA-R	gaaacggcttcagttaaaggggtttcattgccagtgcacttgcgc
OL-KpSP-F	ggaattccatatgagtcgccgtgcacc
OL-KpcpcA-R	gaaacggcttcagttaaaggggtttcatagcgctggcggcctg
OL-cpcB-F	atgtttgatgtgtttaccgcgtgg
OL-cpcB-R	acgcgtcgacactagtttacgccaccgc
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OL-PecpcB-R	ccacgcgggtaaacacatcaaacattgccatggccggctggg
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OL-PhcpcB-R	ccacgcgggtaaacacatcaaacatggcttggtcaccggggt
OL-MacpcB-R	ctcaccacgcgggtaaacacatcaaacattgccagtgcacttgcgct
OL-KpcpcB-R	caccacgcgggtaaacacatcaaacatagcgctggcggcctg

Table S4. Codon optimized gene sequences used in this study

gene	sequences
<i>cpcE</i>	atgagcgaaccgaatctgaacccggcggtatactctggatcaggcgattgcgaacctgcagcagaccgaagatgcgagcg cgcgttattatcgggcgtggtggattggtcgttttcgcggcgcaacctgaaactattgcggcgctgttagtggcgctgga agatgaaaccgatcgagcccggatggtggtatccgctcgcgcgaatgcggcaaaagcgttaggcaaaactgggcgat cgtaagtgtgcccggcgttaattaaagcgtggagtgcaagattattatgtcgcgaaaagcgagcacaggcgtagaa ggcttaggtgatgcacgtgcaatggcggcgctgatggcgaaattaaccggcggttagcggcgcgcaattagtgaag gcaaacctcatctggcgagccgtatgaagcgattatgaagcgctgggcaccttacaagcgggtgaagcattggcctga ttgaaccgtttctggaacattttagcccgaagtgagtgatgcggcgcgctgctgtatttcagttaaccggcgataaccg ctatggcgatctgctgattaccggttagcggtaccgatttacagttacgccgcagcgcgatgatggafttaggtgcgacc ggctatttacctggcgcgcaagcgattgcaaaagcgtttgcggaaaacagcctgaaactgattgcgctgcgcgattatgg gcgacccatcgtaacgtcaagcgagcagcgaaagcaaacggttaagccctgcgagccgtcagattctggaactgatgg atagcctgctgtaa
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