

Supplementary Material

Engineering and modulating functional cyanobacterial CO₂-fixing organelles

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Supplementary Figures and Tables



Supplementary Figure 1. Raw SDS-PAGE gel image of the total cell extracts of the recombinant *E. coli* pLFbC901 and the *E. coli* WT cells. The sample lanes of protein ladder, WT and pLFbC901 were selected to be shown in Figure 1C.

| A | CbbL RbcL | MAVKKYSAGVKEYRQTYWMPEYTPLDSDILACFKITPQPGVDREEAAAAVAAESST MPKTQSAAGYKAGVKDYKLTYYTPDYTPKDTDLLAAFRFSPQPGVPADEAGAAIAAESST :. *.****:*: **: *:*** *:*:************ |
|---|--------------|--|
| | CbbL RbcL | GTWTTVWTDLLTDMDYYKGRAYRIEDVPGDDAAFYAFIAYPIDLFEEGSVVNVFTSLVGN GTWTTVWTDLLTDMDRYKGKCYHIEPVQGEENSYFAFIAYPLDLFEEGSVTNILTSIVGN ************** ***:.*:** * *:: :::******** |
| | CbbL | VFGFKAVRGLRLEDVRFPLAYVKTCGGPPHGIOVERDKMNKYGRPLLGCTIKPKLGLSAK |
| | RbcL | VFGFKAIRSLRLEDIRFPVALVKTFQGPPHGIQVERDLLNKYGRPMLGCTIKPKLGLSAK *****:*.****:***:* *** **************** |
| | CbbL | NYGRAVYECLRGGLDFTKDDENINSQPFMRWRDRFLFVQDATETAEAQTGERKGHYLNVT |
| | RbcL | NYGRAVYECLRGGLDFTKDDENINSQPFQRWRDRFLFVADAIHKSQAETGEIKGHYLNVT |
| | | |
| | CbbL | APTPEEMYKRAEFAKEIGAPIIMHDYITGGFTANTGLAKWCQDNGVLLHIHRAMHAVIDR |
| | RbcL | APTCEEMMKRAEFAKELGMPIIMHDFLTAGFTANTTLAKWCRDNGVLLHIHRAMHAVIDR *** *** *******:* *****::*.************ |
| | CbbL | NPNHGIHFRVLTKILRLSGGDHLHTGTVVGKLEGDRASTLGWIDLLRESFIPEDRSRGIF |
| | RbcL | QRNHGIHFRVLAKCLRLSGGDHLHSGTVVGKLEGDKASTLGFVDLMREDHIEADRSRGVF : ********: ********:***************** |
| | CbbL | FDQDWGSMPGVFAVASGGIHVWHMPALVNIFGDDSVLQFGGGTLGHPWGNAAGAAANRVA |
| | RbcL | FTQDWASMPGVLPVASGGIHVWHMPALVEIFGDDSVLQFGGGTLGHPWGNAPGATANRVA * ***.****:.*************************** |
| | CbbL | LEACVEARNQGRDIEKEGKEILTAAAQHSPELKIAMETWKEIKFEFDTVDKLDTQNR |
| | RbcL | LEACVQARNEGRDLYREGGDILREAGKWSPELAAALDLWKEIKFEFETMDKL ****:***:***::*:*** |
| R | CcmK2 | MPIAVGMIETLGFPAVVEAADAMVKAARVTLVGYEKIGSGRVTVIVRGDVSEVOAS |
| U | CsoS1A | MADVTGIALGMIETRGLVPAIEAADAMTKAAEVRLVGRQFVGGGYVTVLVRGETGAVNAA |
| | PduA | MQQEALGMVETKGLTAAIEAADAMVKSANVMLVGYEKIGSGLVTVIVRGDVGAVKAA *:**:** *: .:************************** |
| | CcmK2 | VSAGLDSAKRVAGGEVLSHHIIARPHENLEYVLPIRYTEAVEQFRM |
| | CsoS1A | VRAGADACERVGDG-LVAAHIIARVHSEVENILPKAPQA |
| | PduA | TDAGAAAARNVGEVKAVHVIPRPHTDVEKILPKGISQ |
| | | · ** ···· * · * *** * * ·** |

Supplementary Figure 2. Sequence alignment of representative BMC proteins using Clustal Omega multiple sequence alignment program. (A) Sequence similarity (78%) between the Form 1B Rubisco large subunit CbbL from *H. neapolitanus* and the Form 1B Rubisco large subunit RbcL from Syn7942. (B) Sequence conservation of representative BMC shell hexamers, CcmK2 from Syn7942, CsoS1A from *H. neapolitanus* and PduA from *S. Typhimurium* (CsoS1A – CcmK2: 53%; PduA – CcmK2: 61%).

| Primers | Sequences | Description | | | |
|------------------|--|--|--|--|--|
| Long fragment F | CCCCTGCCAGTCTCTTGATTAAGGTTTGCTTCTTGCTAGAGAC | | | | |
| Long fragment R | TCAACATATTCTACTATCAAGACAAATCAGGCTTTAG | For pLFbC901- pETM11 | | | |
| Short fragment F | TTTGTCTTGATAGTAGAATATGTTGATGGCTCTTC | | | | |
| Short fragment R | rt fragment R ATCTCAGTGGTGGTGGTGGTGGTGGTGCAATCAACGTGTTGAACAATTTC | | | | |
| 6k F | CCCCTGCCAGTCTCTTCTGATTAAG GTTTGCTTCTTGCTAGAGAC | | | | |
| 6k R | ATCTCAGTGGTGGTGGTGGTGGTGGTGCCTATCAAGACAAATCAGGCTTT AG | For pLFbC601- pETM11 | | | |
| RbcL F | ACAGGAAACAGCGATGCCCAAGACGCAATCTGCC | | | | |
| RbcL R | GCCTGCAGGTCGACTTTAGTAGCGGCCGGGACG | | | | |
| ccmK4 F | GCGCGAATTCGATGTCTCAGCAGGCAATTGG | | | | |
| ccmK4 R | GCGCGGTACCATTCCGGGGATCCGTCGACC | For <i>ccmK4</i> - <i>egfp</i> -pTTQ18 | | | |
| b-cb-pAM2991 F | AGGAAACAGACCATGGGTTTGCTTCTTGCTAGAG | For pLFbC901- | | | |
| b-cb-pAM2991 R | CAGGTCGACTCTAGAGAATCAACGTGTTGAACAATTTC | express in Syn7942 | | | |
| CsoS1A F | AGGAAACAGACCATGGAGGCTTGTTCCGGTTCTTTG | | | | |
| CsoS1A R | CTCCGGGCCCGGCAGTTAGGCTTGTGGCGCCTT | For CsoS1A- CFP- pAM2991 express in Sym7942 | | | |
| CFP F | CTGCCGGGCCCGGAGCTG | | | | |
| CFP R | CAGGTCGACTCTAGAG TTAAGCGCTTACGTAGAGC | Syn/942 | | | |
| CbbL F | AGGAAACAGACCATGGATGGCAGTTAAAAAGTATAGTG | | | | |
| CbbL R | CTCCGGGCCCGGCAGTCAACGATTTTGAGTGTCGA | For CbbL-YFP- pAM2991 | | | |
| YFP F | CTGCCGGGCCCGGAGCTG | express in | | | |
| YFP R | CAGGTCGACTCTAGAGTTACTTGTACAGCTCGTC | 5yn/942 | | | |
| pduA F | ACACAGGAAACAGACCATGGCCCAACTATCGGAACACTC | | | | |
| pduA R | GGAGCCAGCGGATCCTTGGCTAATTCCCTTCGGTA | For PduA- sfGFP- | | | |
| sfGFP F | F GCCAAGGATCCGCTGGCTCCGCTGC p4 ex | | | | |
| sfGFP R | CCTGCAGGTCGACTCTAGAGAAGGAGGATATTCATATGTTATTTG | Syn7942 | | | |

Supplementary Table 1. Oligonucleotides used in cloning.

| CyanoBase | UniProt | Protein Description | Masco t score | Coverage (%) | Unique peptides |
|-----------------|---------|--------------------------------|---------------------|-----------------|----------------------|
| | Q31NB3 | Rubisco large chain RbcL | 206 | 14 | KLTYYTPDYTPKD |
| | | | | | KDTDLLAAFRF |
| Same 22042 1426 | | | | | RAVYECLRG |
| Synpcc/942_1420 | | | | | KASTLGFVDLMRE |
| | | | | | REDHIEADRS |
| | | | | | KWSPELAAALDLWKE |
| | Q31NB2 | Rubisco small chain RbcS | 178 | 42 | RFETFSYLPPLSDRQ |
| | | | | | KLPLFDCKS |
| Synpcc7942_1427 | | | | | KSPQQVLDEVRE |
| | | | | | RSEYGDCYIRV |
| | | | | | RVAGFDNIKQ |
| | Q03511 | CcmK2 | 155 | 40 | RVTLVGYEKI |
| S | | | | | RVTVIVRG |
| Synpcc/942_1421 | | | | | RGDVSEVQASVSAGLDSAKR |
| | | | | | RYTEAVEQFRM |
| | Q03513 | CcmM | 129 | 6 | RLSSEVITQVRS |
| Synpcc7942_1423 | | | | | RNLLAQGYRI |
| | | | | | RVFEALIQDPQGPVGSAKA |
| | Q31RK2 | CcmK4 | 112 | 24 | RFAVNIRG |
| Synpcc7942_0285 | | | | | KTAMDAGIEAAKN |
| | | | | | RLSAEGTGSGRR |

Supplementary Table 2. Proteomic analysis of identified β-carboxysome proteins expressed by pLFbC901 in the whole *E. coli* cell extract.