Supplementary data

Supplementary Table S1: Primers and antibiotics used in this work. Restriction site that were added to the gene-specific primers are underlined.

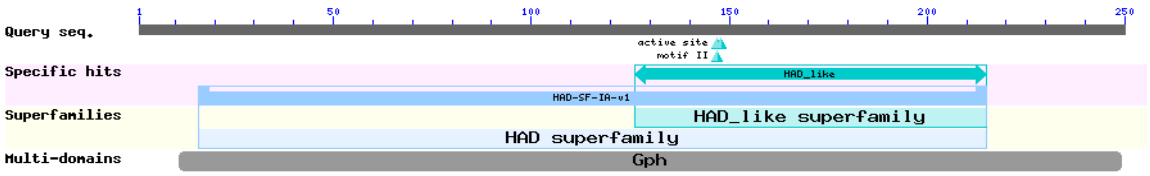
Primers used for generation of *Synechocystis* mutants			
Gene	**Primer**	**Antibiotic**	**Dosage**
slr0458	Fw_CCCAACTTATCCCAAAGATA Rev_GCCGGAATTAGATTTACAAG	Chloramphenicol (Cm)	15 µg ml^{-1}
slr0586	Fw_CCCCTACAGCATTGACCGCAACATT Rev_GGCAAACATTGGGTTCATCGGCTT	Erythromycin (Ery)	100 µg ml^{-1}
sll1349	Fw_AGATTTACAGAGCCHTTATT Rev_GATTGAGCCAATATTTGAGG	Kanamycin (Kan)	50 µg ml^{-1}
slr1762	Fw_AGGGCAAGTTAGCGGAGTT Rev_AAGGGCTATCTTCCCACGTT	Spectinomycin (Sp)	20 µg ml^{-1}
Primers used for cloning in *E. coli* pET28a vector			
slr0458	Fw_AACATATGGCTAACGAAAAAATTATTG Rev_TTGAATTCTAAAGGGCGATCGCC		
slr0586	Fw_AACATATGGCTAATATGGTGCAGAAG Rev_TTGAATTCTATTCAACCCCATGG		
sll1349	Fw_AACATATGGCCATTAAAGCTGTATTG Rev_TTGAATTCTAGGATTTTAATGG		
slr1762	Fw_AACATATGGCTAATATCACCGTTCAAG Rev_TTGAATTCTTAGGGCTGGCAGTG		

Supplementary Table S2: Identity and similarity matrix of *Synechocystis* PGPase candidate proteins. Similarities were given in brackets and calculated based on the Blosum62 substitution matrix.

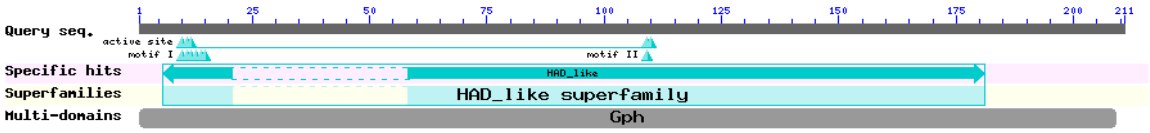
	Slr0458	Sll1349	Slr1762	Slr0586	AtPGLP1
Slr0458	100%				
Sll1349	20(65)%	100%			
Slr1762	19(54)%	17(54)%	100%		
Slr0586	20(56)%	23(58)%	(17(48)%	100%	
AtPGLP1	14(29)%	10(24)%	11(23)%	20(32)%	100%

Supplementary Figure S1: Domain structure of putative PGPases

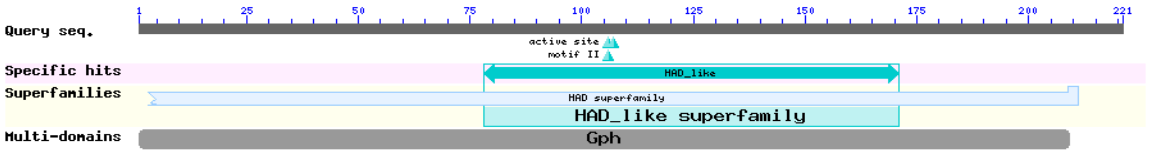
gi|499176709|Synechocystis sp. PCC 6803 slr0458



gi|499176735|Synechocystis sp. PCC 6803 slr0586



gi|499174608|Synechocystis sp. PCC 6803 sll1349



gi|499174101|Synechocystis sp. PCC6803 slr1762

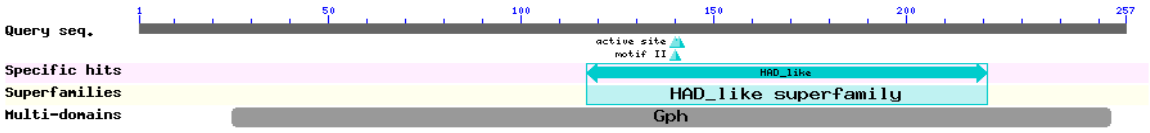


Figure S1: Domain structure including always a HAD-like family domain of the putative PGPases encoded by the ORF's of *slr0458*, *slr0586*, *sll1349,* and *slr1762* (domains were identified via the BLAST server at NCBI).

Supplementary Figure S2: Genotypes of the *Synechocystis* triple mutants

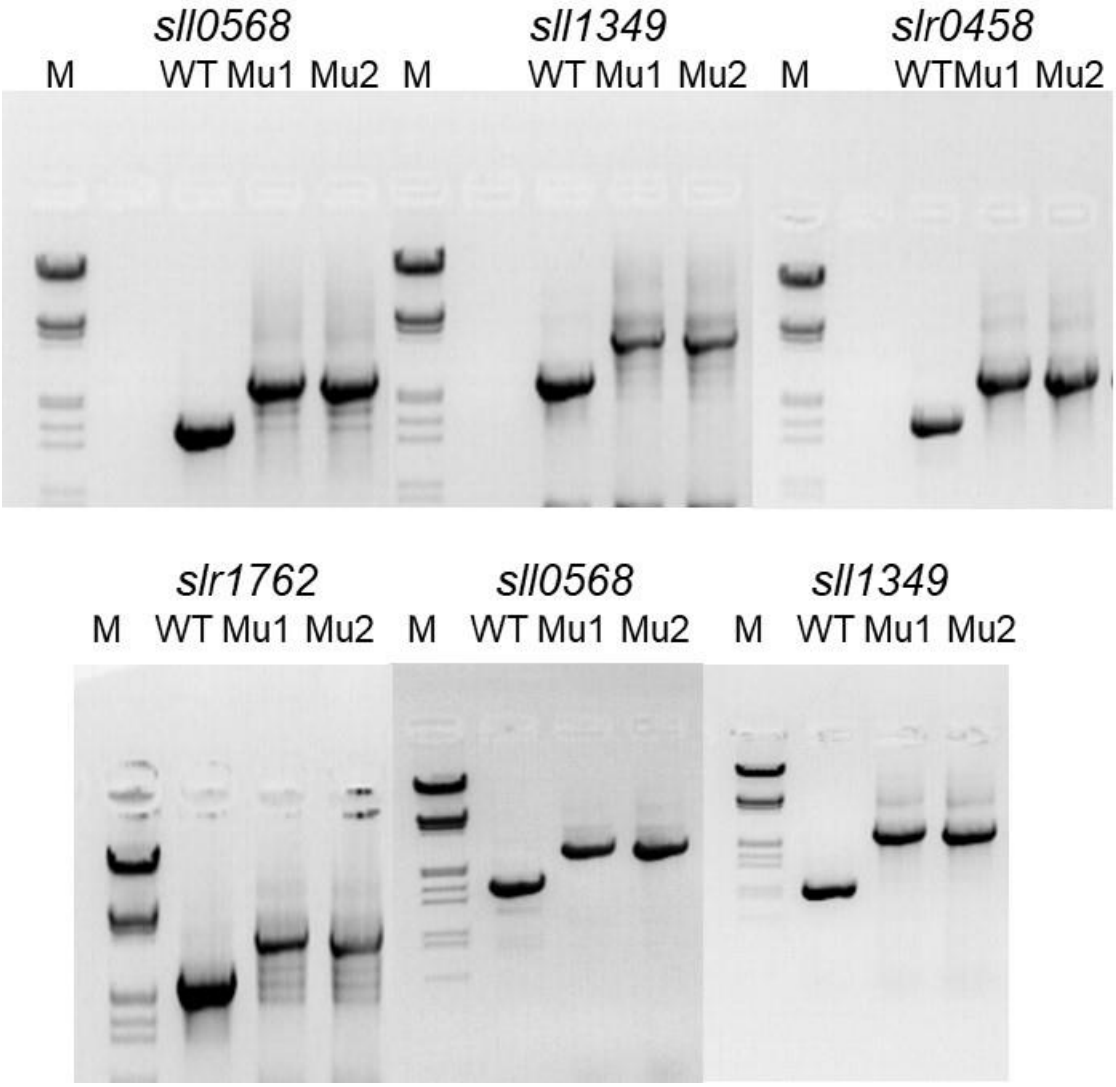


Figure S2: PCR reactions to proof the genotypes of two different triple mutants (upper panel: Δ*sll0568*/*sll1349*/*slr0458*, lower panel Δ*slr1762*/*sll0568*/*sll1349*) of *Synechocystis* defective in three of the four genes encoding for putative PGPases. Gene-specific primers were used (indicated above the pictures) and chromosomal DNA from two mutant clones (Mu1 and Mu2) as well as from wild type (WT) was used as template. M: DNA fragment size marker, λ-DNA cut with *Eco*RI and *Hin*dIII.

Supplementary Figure S3: Growth of *Synechocystis* mutants at ambient air on agar plates

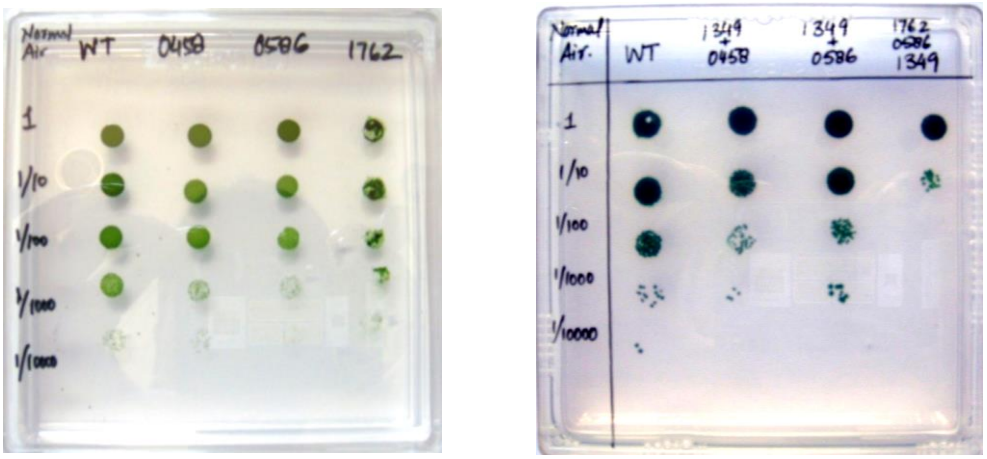


Figure S3: Left panel shows the growth of the *Synechocystis* wild type (WT) and single mutants Δ*slr0458*, Δ*slr0586*, and Δ*slr1762.* Right panel shows the growth of the *Synechocystis* wild type (WT), double mutants Δ*sll1349*/Δ*slr0458*, Δ*sll1349*/Δ*slr0586*, and triple mutant Δ*slr1762*/*slr0586*/*sll1349* (indicated by gene names). Increasingly diluted cell suspensions were dropped (2 µl each) on BG11 agar plates and incubated at 50 µmol photons m^{-2} s^{-1} at 30°C under ambient air conditions for 10 days.

Supplementary Figure S4: Expression and purification of PGPases in *E. coli*

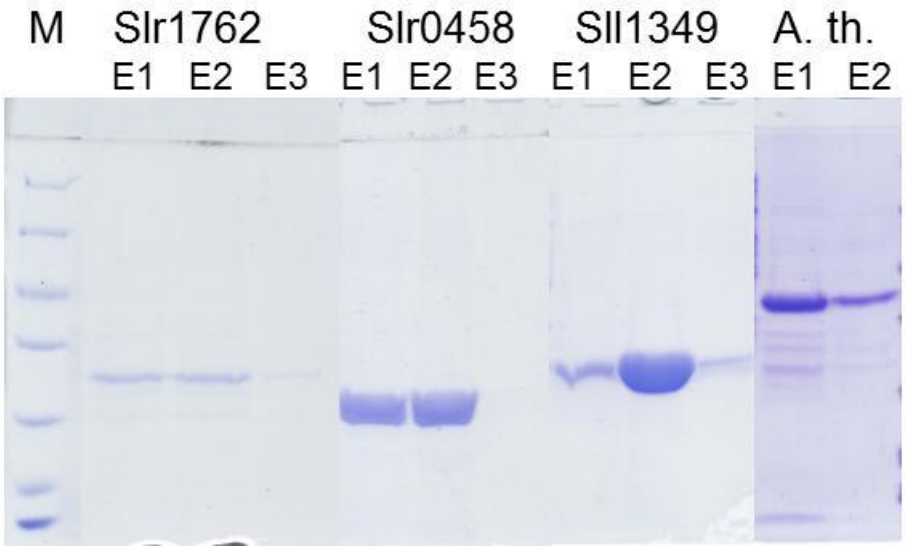
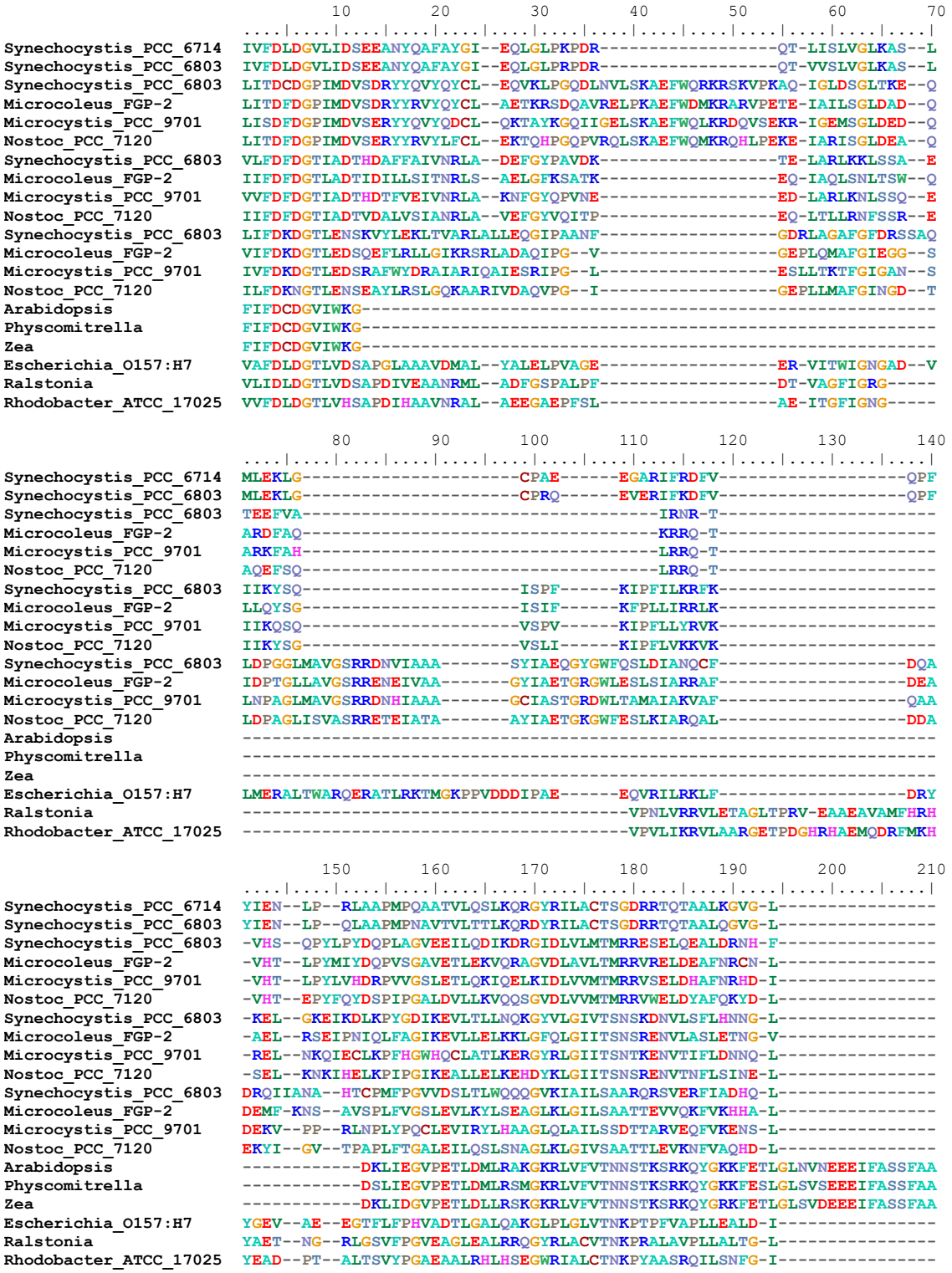
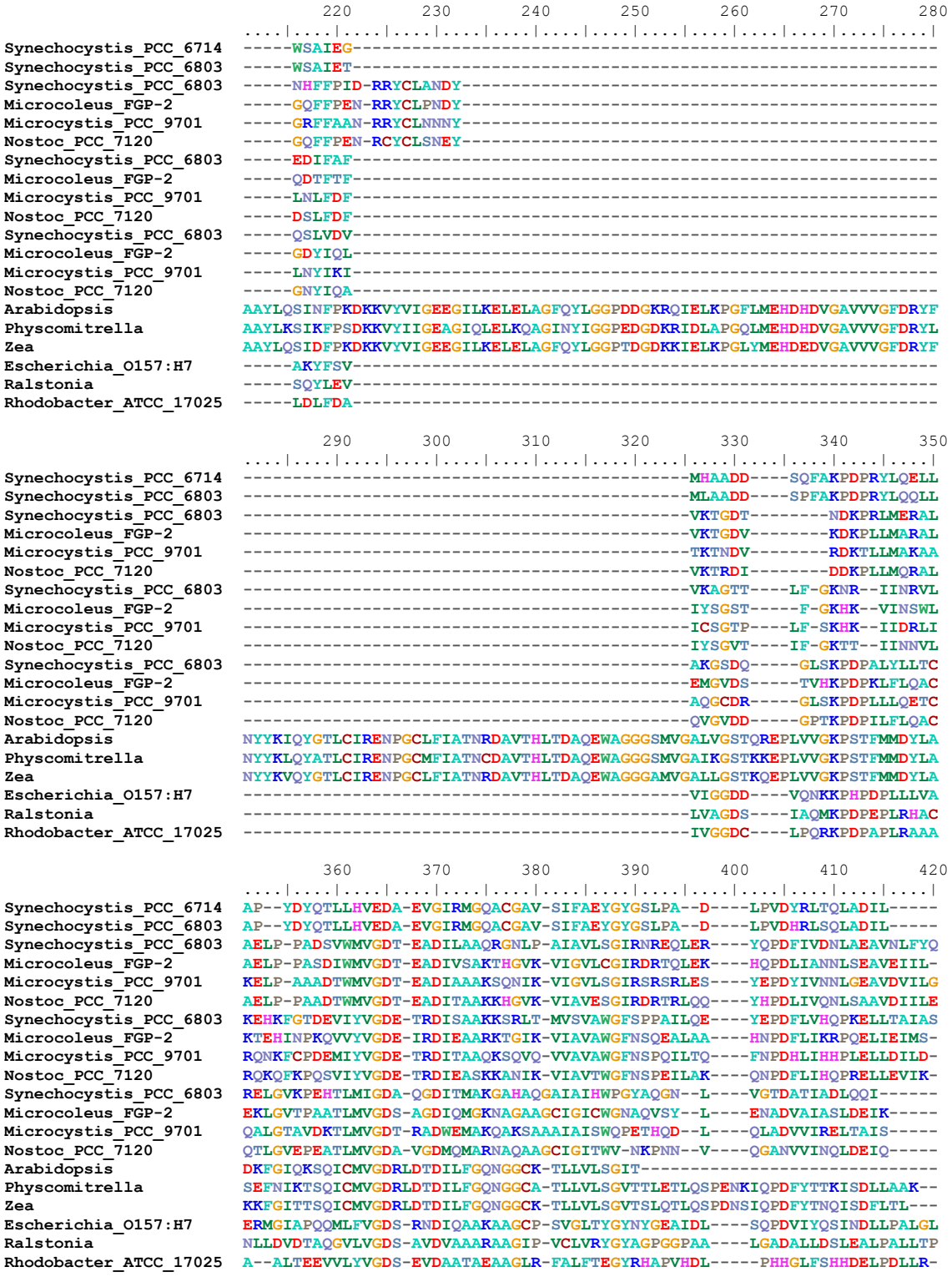


Figure S4: Purity of the recombinant PGPases from *Synechocystis* (Slr1762, Slr0458, Sll1349) and the PGPase from *Arabidopsis thaliana* (A.th.), which were used for the enzyme assays. The SDS-PAGE lanes show elution fractions (E) 1, 2, or 3 that were obtained after purification of the His-tagged proteins from recombinant *E. coli* cells. M: protein size marker (Biorad).

Supplementary Figure S5: Alignment of *Synechocystis* PGPase-like proteins, which was used for phylogenetic reconstruction.

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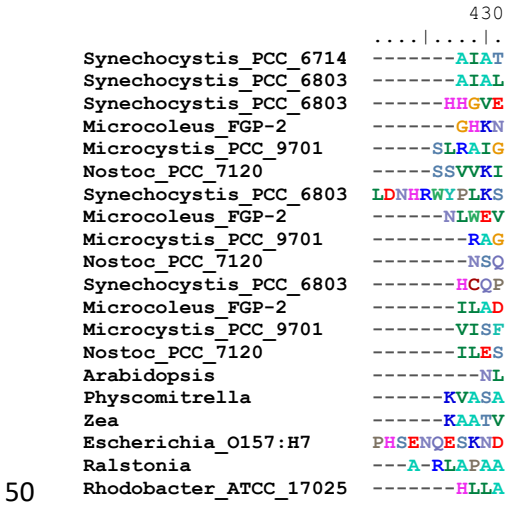
Synechocystis_PCC_6803 -----WSAIET----------
Synechocystis_PCC_6803 -----NHFFPID-RRYCLANDY----------
Microcoleus_FGP-2 -----GQFFPEN-RRYCLPNDY----------
Microcystis_PCC_9701 -----GRFFAAN-RRYCLNNNY----------
Nostoc_PCC_7120 -----GQFFPEN-RCYCLSNEY----------
Synechocystis_PCC_6803 -----EDIFAF----------
Microcoleus_FGP-2 -----QDTFTF----------
Microcystis_PCC_9701 -----LNLFDF----------
Nostoc_PCC_7120 -----DSLFDF----------
Synechocystis_PCC_6803 -----QSLVDV----------
Microcoleus_FGP-2 -----GDYIQL----------
Microcystis_PCC_9701 -----LNYIKI----------
Nostoc_PCC_7120 -----GNYIQA----------
Arabidopsis AAYLQSINFPKDKKVYVIGEEGILKELELAGFQYLGGPDDGKRQIELKPGFLMEHDHDVGAVVVGFDRYF
Physcomitrella AAYLKSIKFPSDKKVYIIGEAGIQLELKQAGINYIGGPEDGDKRIDLAPGQLMEHDHDVGAVVVGFDRYL
Zea AAYLQSIDFPKDKKVYVIGEEGILKELELAGFQYLGGPTDGDKKIELKPGLYMEHDEDVGAVVVGFDRYF
Escherichia_O157:H7 -----AKYFSV----------
Ralstonia -----SQYLEV----------
Rhodobacter_ATCC_17025 -----LDLFDA----------
290 300 310 320 330 340 350
Synechocystis_PCC_6714 ----------MHAADD----SQFAKPDPRYLQELL
Synechocystis_PCC_6803 ----------MLAADD----SPFAKPDPRYLQQLL
Synechocystis_PCC_6803 ----------VKTGDT--------NDKPRLMERAL
Microcoleus_FGP-2 ----------VKTGDV--------KDKPLLMARAL
Microcystis_PCC_9701 ----------TKTNDV--------RDKTLLMAKAA
Nostoc_PCC_7120 ----------VKTRDI--------DDKPLLMQRAL
Synechocystis_PCC_6803 ----------VKAGTT----LF-GKNR--IINRVL
Microcoleus_FGP-2 ----------IYSGST-----F-GKHK--VINSWL
Microcystis_PCC_9701 ----------ICSGTP----LF-SKHK--IIDRLI
Nostoc_PCC_7120 ----------IYSGVT----IF-GKTT--IINNVL
Synechocystis_PCC_6803 ----------AKGSDQ-----GLSKPDPALYLLTC
Microcoleus_FGP-2 ----------EMGVDS-----TVHKPDPKLFLQAC
Microcystis_PCC_9701 ----------AQGCDR-----GLSKPDPLLLQETC
Nostoc_PCC_7120 ----------QVGVDD-----GPTKPDPILFLQAC
Arabidopsis NYYKIQYGTLCIRENPGCLFIATNRDAVTHLTDAQEWAGGGSMVGALVGSTQREPLVVGKPSTFMMDYLA
Physcomitrella NYYKLQYATLCIRENPGCMFIATNCDAVTHLTDAQEWAGGGSMVGAIKGSTKKEPLVVGKPSTFMMDYLA
Zea NYYKVQYGTLCIRENPGCLFIATNRDAVTHLTDAQEWAGGGAMVGALLGSTKQEPLVVGKPSTFMMDYLA
Escherichia_O157:H7 ----------VIGGDD----VQNKKPHPDPLLLVA
Ralstonia ----------LVAGDS----IAQMKPDPEPLRHAC
Rhodobacter_ATCC_17025 ----------IVGGDC----LPQRKPDPAPLRAAA
360 370 380 390 400 410 420
Synechocystis_PCC_6714 AP--YDYQTLLHVEDA-EVGIRMGQACGAV-SIFAEYGYGSLPA--D----LPVDYRLTQLADIL-----
Synechocystis_PCC_6803 AP--YDYQTLLHVEDA-EVGIRMGQACGAV-SIFAEYGYGSLPA--D----LPVDHRLSQLADIL-----
Synechocystis_PCC_6803 AELP-PADSVWMVGDT-EADILAAQRGNLP-AIAVLSGIRNREQLER----YQPDFIVDNLAEAVNLFYQ
Microcoleus_FGP-2 AELP-PASDIWMVGDT-EADIVSAKTHGVK-VIGVLCGIRDRTQLEK----HQPDLIANNLSEAVEIIL-
Microcystis_PCC_9701 KELP-AAADTWMVGDT-EADIAAAKSQNIK-VIGVLSGIRSRSRLES----YEPDYIVNNLGEAVDVILG
Nostoc_PCC_7120 AELP-PAADTWMVGDT-EADITAAKKHGVK-VIAVESGIRDRTRLQQ----YHPDLIVQNLSAAVDIILE
Synechocystis_PCC_6803 KEHKFGTDEVIYVGDE-TRDISAAKKSRLT-MVSVAWGFSPPAILQE----YEPDFLVHQPKELLTAIAS
Microcoleus_FGP-2 KTEHINPKQVVYVGDE-IRDIEAARKTGIK-VIAVAWGFNSQEALAA----HNPDFLIKRPQELIEIMS-
Microcystis_PCC_9701 RQNKFCPDEMIYVGDE-TRDITAAQKSQVQ-VVAVAWGFNSPQILTQ----FNPDHLIHHPLELLDILD-
Nostoc_PCC_7120 RQKQFKPQSVIYVGDE-TRDIEASKKANIK-VIAVTWGFNSPEILAK----QNPDFLIHQPRELLEVIK-
Synechocystis_PCC_6803 RELGVKPEHTLMIGDA-QGDITMAKGAHAQGAIAIHWPGYAQGN--L----VGTDATIADLQQI------
Microcoleus_FGP-2 EKLGVTPAATLMVGDS-AGDIQMGKNAGAAGCIGICWGNAQVSY--L----ENADVAIASLDEIK-----
Microcystis_PCC_9701 QALGTAVDKTLMVGDT-RADWEMAKQAKSAAAIAISWQPETHQD--L----QLADVVIRELTAIS-----
Nostoc_PCC_7120 QTLGVEPEATLMVGDA-VGDMQMARNAQAAGCIGITWV-NKPNN--V----QGANVVINQLDEIQ-----
Arabidopsis DKFGIQKSQICMVGDRLDTDILFGQNGGCK-TLLVLSGIT----------
Physcomitrella SEFNIKTSQICMVGDRLDTDILFGQNGGCA-TLLVLSGVTTLETLQSPENKIQPDFYTTKISDLLAAK--
Zea KKFGITTSQICMVGDRLDTDILFGQNGGCK-TLLVLSGVTSLQTLQSPDNSIQPDFYTNQISDFLTL---
Escherichia_O157:H7 ERMGIAPQQMLFVGDS-RNDIQAAKAAGCP-SVGLTYGYNYGEAIDL----SQPDVIYQSINDLLPALGL
Ralstonia NLLDVDTAQGVLVGDS-AVDVAAARAAGIP-VCLVRYGYAGPGGPAA----LGADALLDSLEALPALLTP
Rhodobacter_ATCC_17025 A--ALTEEVVLYVGDS-EVDAATAEAAGLR-FALFTEGYRHAPVHDL-----PHHGLFSHHDELPDLLR-

49


430
....|....|.
Synechocystis_PCC_6714 -------AIAT
Synechocystis_PCC_6803 -------AIAL
Synechocystis_PCC_6803 ------HHGVE
Microcoleus_FGP-2 -------GHKN
Microcystis_PCC_9701 -----SLRAIG
Nostoc_PCC_7120 -----SSVVKI
Synechocystis_PCC_6803 LDNHRWYPLKS
Microcoleus_FGP-2 ------NLWEV
Microcystis_PCC_9701 --------RAG
Nostoc_PCC_7120 --------NSQ
Synechocystis_PCC_6803 -------HCQP
Microcoleus_FGP-2 -------ILAD
Microcystis_PCC_9701 -------VISF
Nostoc_PCC_7120 -------ILES
Arabidopsis ---------NL
Physcomitrella ------KVASA
Zea ------KAATV
Escherichia_O157:H7 PHSENQESKND
Ralstonia ---A-RLAPAA
Rhodobacter_ATCC_17025 -------HLLA