

Supplementary data

Supplementary Table S1: Primers and antibiotics used in this work.

Supplementary Table S2: Similarities of cyanobacterial and *Arabidopsis* PGPase proteins

Supplementary Figure S1: Domain structure of putative PGPases

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

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

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

Supplementary Figure S5: Alignment of *Synechocystis* PGPase-like proteins

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

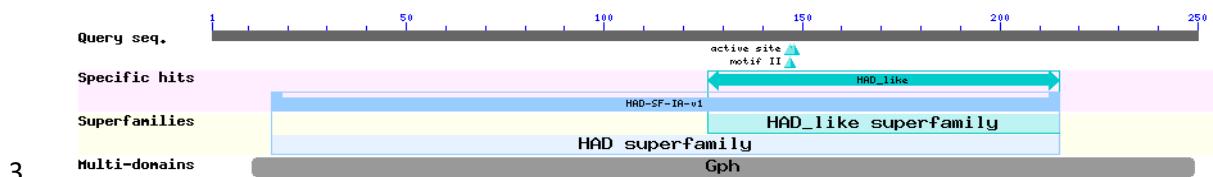
Primers used for generation of <i>Synechocystis</i> mutants			
Gene	Primer	Antibiotic	Dosage
<i>slr0458</i>	Fw_CCCAACTTATCCCAAAGATA Rev_GCCGGAATTAGATTACAAG	Chloramphenicol (Cm)	15 µg ml ⁻¹
<i>slr0586</i>	Fw_CCCCTACAGCATTGACCGAACATT Rev_GGCAAACATTGGGTTCATCGGCTT	Erythromycin (Ery)	100 µg ml ⁻¹
<i>sll1349</i>	Fw_AGATTACAGAGCCHTTATT Rev_GATTGAGCCAATATTGAGG	Kanamycin (Kan)	50 µg ml ⁻¹
<i>slr1762</i>	Fw_AGGGCAAGTTAGCGGAGTT Rev_AAGGGCTATCTTCCCACGTT	Spectinomycin (Sp)	20 µg ml ⁻¹
Primers used for cloning in <i>E. coli</i> pET28a vector			
<i>slr0458</i>	Fw_A <u>ACATATGG</u> CTAACGAAAAAATTATTG Rev_T <u>TG</u> AATTCTAAAGGGCGATCGCC		
<i>slr0586</i>	Fw_A <u>ACATATGG</u> CTAATATGGTGAGAAG Rev_T <u>TG</u> AATTCTATTCAACCCCCATGG		
<i>sll1349</i>	Fw_A <u>ACATATGG</u> CCATTAAAGCTGTATTG Rev_T <u>TG</u> AATTCTAGGATTTAATGG		
<i>slr1762</i>	Fw_A <u>ACATATGG</u> CTAATATCACCGTTCAAG Rev_T <u>TG</u> AATTCTAGGGCTGGCAGTG		

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	SII1349	Slr1762	Slr0586	AtPGLP1
Slr0458	100%				
SII1349	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%

1 **Supplementary Figure S1: Domain structure of putative PGPases**

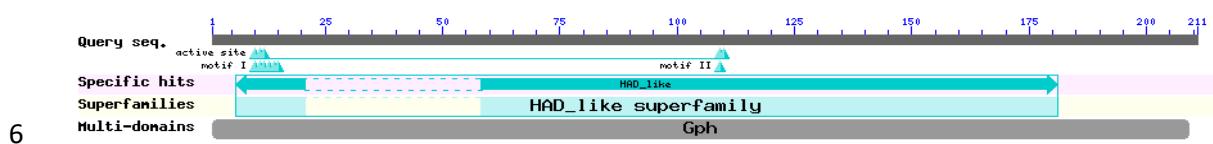
2 gi|499176709|Synechocystis sp. PCC 6803 slr0458



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4

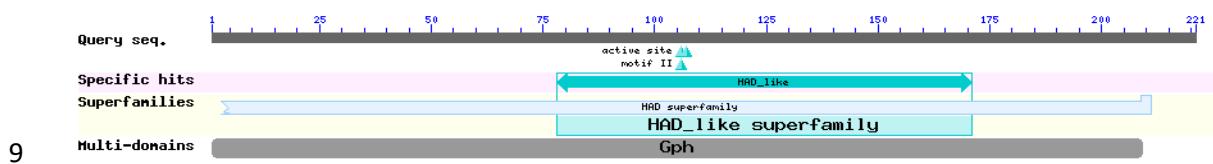
5 gi|499176735|Synechocystis sp. PCC 6803 slr0586



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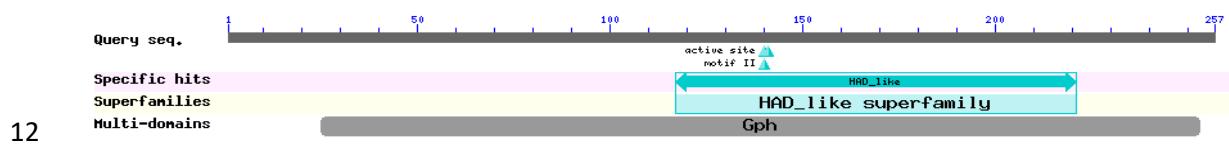
8 gi|499174608|Synechocystis sp. PCC 6803 sll1349



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11 gi|499174101|Synechocystis sp. PCC6803 slr1762

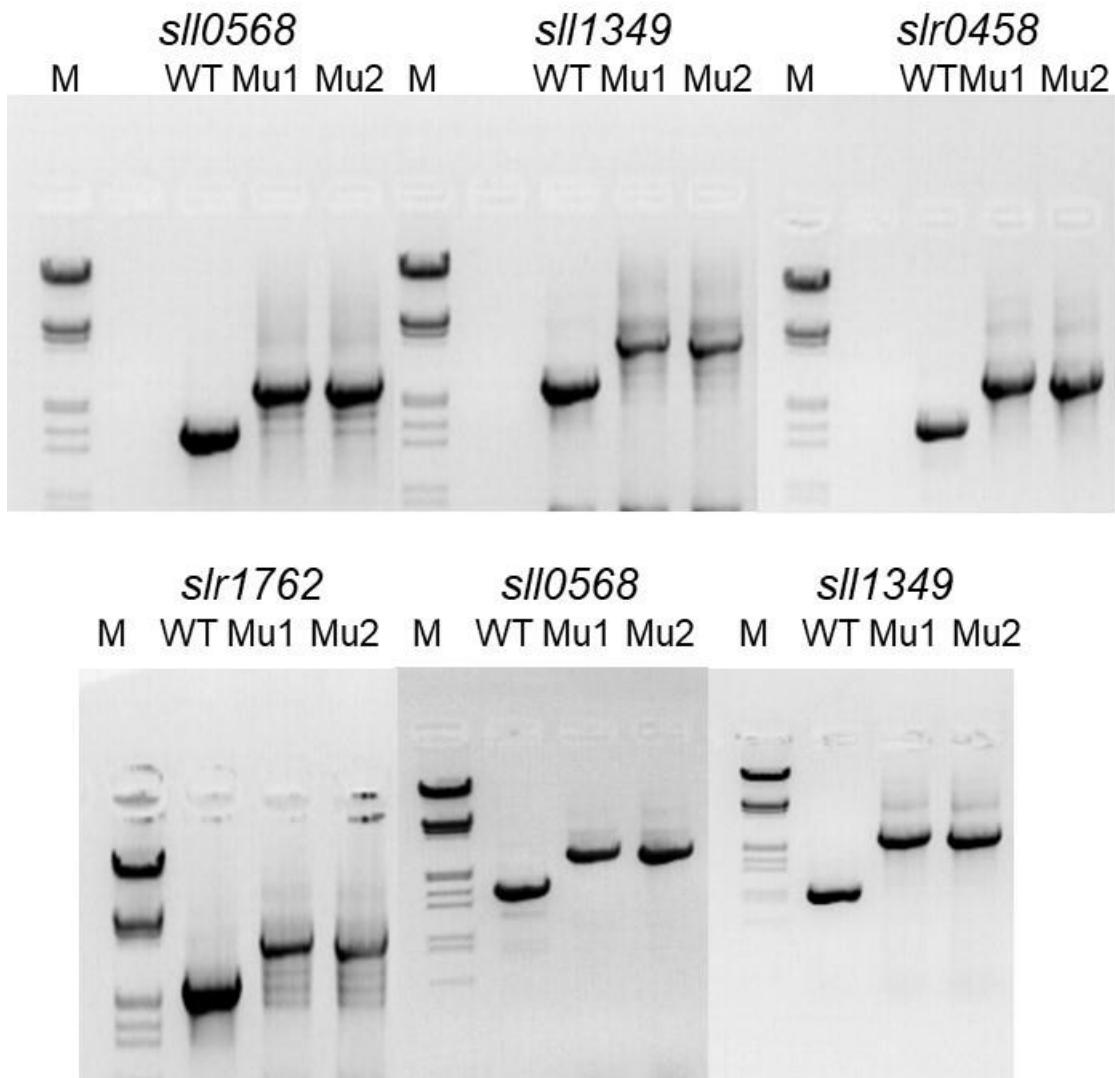


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13 **Figure S1:** Domain structure including always a HAD-like family domain of the putative
14 PGPases encoded by the ORF's of *slr0458*, *slr0586*, *sll1349*, and *slr1762* (domains were
15 identified via the BLAST server at NCBI).

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

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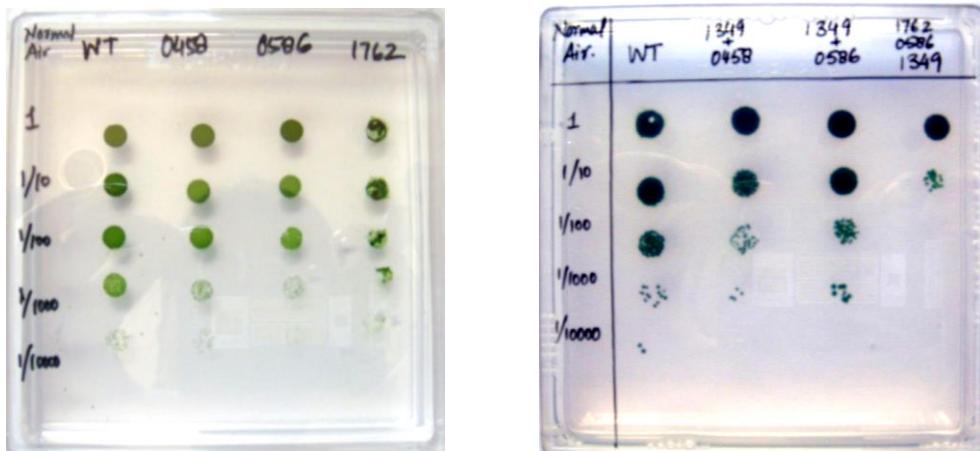
18

19 **Figure S2:** PCR reactions to proof the genotypes of two different triple mutants (upper
20 panel: $\Delta sll0568/sll1349/sl0458$, lower panel $\Delta slr1762/sll0568/sll1349$) of *Synechocystis*
21 defective in three of the four genes encoding for putative PGPases. Gene-specific primers
22 were used (indicated above the pictures) and chromosomal DNA from two mutant clones
23 (Mu1 and Mu2) as well as from wild type (WT) was used as template. M: DNA fragment size
24 marker, λ -DNA cut with *Eco*RI and *Hind*III.

25

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

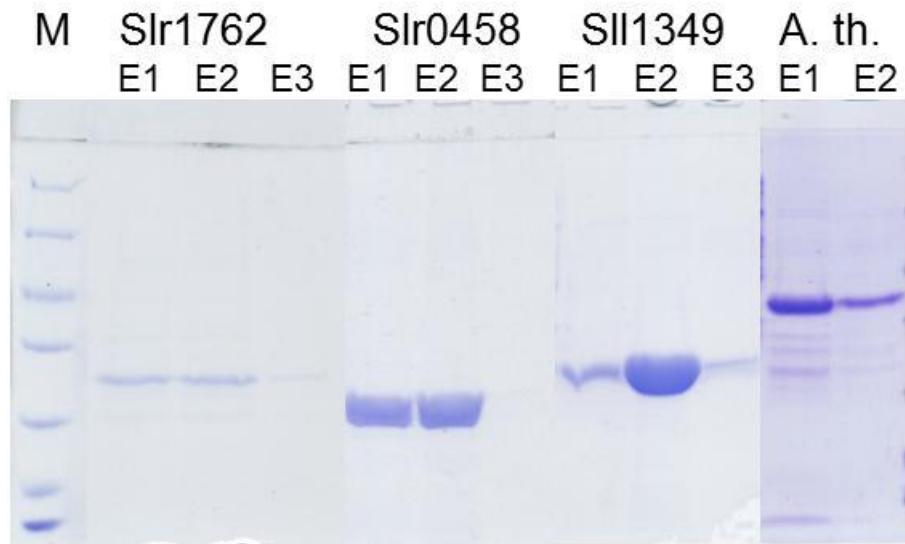
28



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

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

38

39 **Figure S4:** Purity of the recombinant PGPases from *Synechocystis* (Slr1762, Slr0458,
40 SII1349) and the PGPase from *Arabidopsis thaliana* (A.th.), which were used for the
41 enzyme assays. The SDS-PAGE lanes show elution fractions (E) 1, 2, or 3 that were
42 obtained after purification of the His-tagged proteins from recombinant *E. coli* cells. M:
43 protein size marker (Biorad).

44

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

	10	20	30	40	50	60	70	
<i>Synechocystis_PCC_6714</i>	IVF D GVLIDSE E ANYQAFAYGI--EQLGLEKPDR-----	-QT-LISLVGLKAS--L						
<i>Synechocystis_PCC_6803</i>	I V E D LGVLIDSE E ANYQAFAYGI--EQLGLEKPDR-----	-QT-VVSLVGLKAS--L						
<i>Synechocystis_PCC_6803</i>	LITCDGPIMDVSDRYYQVYQCL--EQVKLPGQDNLVLSKA E F W QRKRSKVPKA Q -IGLD S GLTKE--Q							
<i>Microcoleus_FGP-2</i>	LIT D FGPIMDVSDRYYR V YQCL--AE T KRS D QAVREL P KAE F WD M KRA R PETE--IAILSGLDAD--Q							
<i>Microcystis_PCC_9701</i>	LISDFDGPIMDVSE R YQVYQDCL--QKTAYKGQIIGELSKA E FW Q LKR D QVS E KR-IGEMSG I DDED--Q							
<i>Nostoc_PCC_7120</i>	LIT D FDGPIMDVSE R YR V YQCL--EKTQH P GQPVRQLSKA E FW Q MR Q H P EKE--IARISGLDEA--Q							
<i>Synechocystis_PCC_6803</i>	VLF D FGTIADTHDAFFAIVNRLA--DEFGYPAVDK-----TE-LARLKKLSSA--E							
<i>Microcoleus_FGP-2</i>	IIFDFDGT L TD T IDSITNRLS--AELGFKSATK-----EQ-IAQLSNLT S W--Q							
<i>Microcystis_PCC_9701</i>	VVF D FGTIADTHDTFVEIVNRLA--KNFGYQPVNE-----ED-LARLKNLSSQ--E							
<i>Nostoc_PCC_7120</i>	IIFDFDGTIADTVDALVSIANRLA--VEFGYVQITP-----EQ-LTLLRN F SSR--E							
<i>Synechocystis_PCC_6803</i>	LIFKDGTLENSKVY E KL T VAR A LL E QG I FAANF-----GDRLAGAF G EDRSSAQ							
<i>Microcoleus_FGP-2</i>	VIFDKDGTLEDSQEFLR L LG K RSRLADAQ I PG--V-----GEPLQMAF G IEGG--S							
<i>Microcystis_PCC_9701</i>	IVFD D GTL D LSRAFWYDRAIARIQAI E SR I PG--L-----ESLLTKTF G IGAN--S							
<i>Nostoc_PCC_7120</i>	I L F D KN G TLENS E AYL R SLGQKA R IVDAQ V PG--I-----GEPLLMAF G INGD--T							
<i>Arabidopsis</i>	FIFDCDGVIWKG-----							
<i>Physcomitrella</i>	FIFDCDGVIWKG-----							
<i>Zea</i>	FIFDCDGVIWKG-----							
<i>Escherichia_O157:H7</i>	VAF D LDGTLVDSAPGLAAAVDMAL--YALELEVAGE-----ER-VITWI G NCAD--V							
<i>Ralstonia</i>	VL I IDGTLVDSAPDIVEAANRML--ADFGSPALPF-----DT-VAGFIGRG-----							
<i>Rhodobacter_ATCC_17025</i>	VVF D LDGTLVHSAPDIHA A AVNRLA--AEEGAEPFSL-----AE-ITGFIGNG-----							
		80	90	100	110	120	130	140
<i>Synechocystis_PCC_6714</i>	MLEKLG-----CPAE-----EGARIFRD F V-----QPF							
<i>Synechocystis_PCC_6803</i>	MLEKLG-----CPRQ-----EVERIFKD F V-----QPF							
<i>Synechocystis_PCC_6803</i>	TEEFVA-----IRNR-T-----							
<i>Microcoleus_FGP-2</i>	ARDFAQ-----KRRQ-T-----							
<i>Microcystis_PCC_9701</i>	ARKFAQ-----LRRQ-T-----							
<i>Nostoc_PCC_7120</i>	AQEFSQ-----LRRQ-T-----							
<i>Synechocystis_PCC_6803</i>	IIKYSQ-----ISPF-----KIPFILKRFK-----							
<i>Microcoleus_FGP-2</i>	LLQYSG-----ISIF-----KFP L IRR L K-----							
<i>Microcystis_PCC_9701</i>	IIKQSQ-----VSPV-----KIPFILLYRVK-----							
<i>Nostoc_PCC_7120</i>	IIKYSG-----VSLL-----KIPF L VKKVK-----							
<i>Synechocystis_PCC_6803</i>	LDPGGLMAVGSRDNVIAAA--SYIAE Q GYGW Q SLDIANQCF-----DQA							
<i>Microcoleus_FGP-2</i>	IDPTCLLAVGSRRENEIVAA--GYIAETGR G WLESLSIARRAF-----DEA							
<i>Microcystis_PCC_9701</i>	LNPAGLMAVGSRDNHIAAA--GCIASTGRDWLTAMAI A KA V AF-----QAA							
<i>Nostoc_PCC_7120</i>	LDPAGLISVASRRETEIATA--AYIAETGK G WFESLK I ARQAL-----DDA							
<i>Arabidopsis</i>	-----							
<i>Physcomitrella</i>	-----							
<i>Zea</i>	-----							
<i>Escherichia_O157:H7</i>	LMERALTWARQERATL R KT M G K PPV DD D I PAE-----EQR V ILRKLF-----DRY							
<i>Ralstonia</i>	-----VPNLVRRVLETAGLTPRV-EAAEA V AMFHRH-----							
<i>Rhodobacter_ATCC_17025</i>	-----VPVLIKRVLA A RC E TPD G HR H AE M QDRFMKH-----							
		150	160	170	180	190	200	210
<i>Synechocystis_PCC_6714</i>	YIEN--LP--R L A P M P Q A AT V LQ S KLQ R GYR I ACT S G D R R TQ A ALKG V G-----L							
<i>Synechocystis_PCC_6803</i>	YIEN--LP--Q L A P M P NA V T V L T LKQ R GYR I ACT S G D R R TQ A ALKG V G-----L							
<i>Synechocystis_PCC_6803</i>	-VHS--Q P YLPYDQ P LAG V E I ILQ D IK R G I D L V L M T M R R E SELQ E ALDR N H-F-----							
<i>Microcoleus_FGP-2</i>	-VHT--LPYMTYDQ P V S CA V E T LEK V Q R AG V D L V L T M R V R E LE D AF N R C N-L-----							
<i>Microcystis_PCC_9701</i>	-VHT--LPYL V H R P V V G S L ET L Q K IQ E L K IDL V V M T M R V S E LD H AF N R D -I-----							
<i>Nostoc_PCC_7120</i>	-VHT--EPYFYQ D S P I P G A L D V L K V QQ S GV D L V V M T M R V W E LD Y AF Q KYD-L-----							
<i>Synechocystis_PCC_6803</i>	-KEL--GKE I DLK P Y C DI K E V L T L N Q K G V VL G IV T S N K D N V L F HNN G -L-----							
<i>Microcoleus_FGP-2</i>	-AEL--R S E I P N Q L F A GI K E V EL L E K KL G F Q LG I IT S N R EN V LA S LET N G-----V							
<i>Microcystis_PCC_9701</i>	-REI--NK Q I E CL K P F IC G WH O CL A L K ER G Y R LG I IT S N T K E N V T I FL D NN Q -L-----							
<i>Nostoc_PCC_7120</i>	-SEL--K N K I HE L K P IP G IK E AL L E K H D Y K LG I IT S N R EN V NT F L S INE-L-----							
<i>Synechocystis_PCC_6803</i>	DRQ I IANA--HTCPM F PG V V D S L TL W QQ G V K I A IL S AA R Q R S V ER F I A D Q -L-----							
<i>Microcoleus_FGP-2</i>	DEM F -K N S--AV S PL F V G S L E V VL K L S E A GL K LG I LS A AT T EV V Q K F V K H A-L-----							
<i>Microcystis_PCC_9701</i>	DEK V --PP--R L N P LY P C Q LE V IR Y L H A G L Q L A IL S SD T TA R V E Q F V K ENS-L-----							
<i>Nostoc_PCC_7120</i>	EKYI--GV--TPA P LT F CA E IL Q LS N A G IK L LG I VS A TT L E V K N F V A Q D-L-----							
<i>Arabidopsis</i>	-----DKL I E G V P ET I D M L R A K G K RL V F V T N N S TS R K Q Y G K F ET L GL N V NE E E I F A S F AA -							
<i>Physcomitrella</i>	-----DSL I E G V P ET I D M L R SM C K R LV F V T N S TS R K Q Y G K F ET L GL S V SE E E I F A S F AA -							
<i>Zea</i>	-----DKL I D G V P ET I D L LL R SK G K R LV F V T N S TS R K Q Y G R K ET L GL S V DE E E I F A S F AA -							
<i>Escherichia_O157:H7</i>	YGEV--AE--EGT F LP F PH V AD T LG A Q K G L PL G LV T N K P T P F V A PL L E A D-I-----							
<i>Ralstonia</i>	YAET--NG--RLGSV F PG V EA G LE A LR R Q Y RL A CV T N K P R AL V P L ALT G ------							
<i>Rhodobacter_ATCC_17025</i>	YEAD--PT--ALT S V Y PG A AA L R L H S EG W R I AL C T N K P Y A AS R Q I LS N FG-I-----							

47

48

220 230 240 250 260 270 280
 -----WSAIEG-----
 -----WSAIET-----
 -----NHFFPID-RRYCLANDY-----
 -----CQFFPEN-RRYCLPNY-----
 -----CRFFAAN-RRYCLNNY-----
 -----CQFFPEN-RCYCLSNEY-----
 -----EDIFAF-----
 -----QDTFTF-----
 -----LNLFD-----
 -----DSLDF-----
 -----QLSLVDV-----
 -----CDYIQL-----
 -----LNYIKI-----
 -----CNYIQA-----
 Arabidopsis AAYLQSINFPKDKKKVVVIGEEGILKELELAGFQYLGGPDDGKRQIELKPGLMEHDHDVGAVVVGFDRYF
 Physcomitrella AAYLKSIKFPSDKKKVVVIGEAGIQLELKQAGINYIGGPEDGDKRIDLAPGQLMHDHDVGAVVVGFDRYF
 Zea AAYLQSIDFPKDKKKVVVIGEEGILKELELAGFQYLGGPTDGDKKIELKPGLYMEHDEDVGAVVVGFDRYF
 Escherichia_O157:H7 -----AKYFSV-----
 Ralstonia -----SQYLEV-----
 Rhodobacter_ATCC_17025 -----LDLFDA-----

 290 300 310 320 330 340 350
 -----MHAADD-----SQFAKPPDPRYLQELL
 -----MLAADD-----SPFAKPDPRYLQOLL
 -----VKTCDT-----NDKPERLIMERAL
 -----VKTGDV-----KDKPLLMARAL
 -----TKTNDV-----RDKTLLMAKAA
 -----VKTRDI-----DDKPLLMQRAL
 -----VKRAGTT-----LF-GKNR--IINRVL
 -----IYSGST-----F-GKHK--VINSWL
 -----ICSGTP-----LF-SKHK--IIDRLI
 -----IYSGVT-----IF-GKTT--IINNVL
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 -----AQCCDR-----GLSKPDPLLQETC
 -----QVGVDD-----GPTKPDPLFLQAC
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 -----VIGCDD-----VQNKKPPDPILLVA
 -----LVAGDS-----IAQMKPDPPEPLRHAC
 -----IVGGDC-----LPQRKPDPAPLRAAA

 360 370 380 390 400 410 420
 -----AP-YDYQTLHVEDA-EVGIRMGQACGAV-SIFAEYGYGSLPA---D---LPVDPYRLTQLADIL-----
 -----AP-YDYQTLHVEDA-EVGIRMGQACGAV-SIFAEYGYGSLPA---D---LPVDPHRLSQLADIL-----
 -----AELP-PADSIVWMVGDT-EADILAAQRGNLP-AIAVLSGIRNREQLER---YQPDFIVDNLAEVNLFYQ-----
 -----AELP-PASDIWVMGDT-EADIVSAKTHGVK-VIGVLCGIRDRTQLEK---HQPDLIANNLSEAVEIIL-
 KELP-AAADTWMVGDT-EADIAAAKSQNIK-VIGVLSGIRSRSLES---YEPEYIVNNLGEAVDVILG-----
 -----AELP-PAADTWMVGDT-EADITAAKKHGVK-VIAVESGIRDRTRLQQ---YHPDLIVQNLSAAVDIILE-----
 KEHKFCIDDEVIVYGDE-TRDISAAKSRLT-MVSVAWGFSPAILQE---YEPEFLVHQPFELLTAIAS-----
 KTEHINPKQVYVGDE-IRDIEAARKTGIK-VIAVAWGFNSQEALAA---HNPDFLIKRPQELIEIMS-----
 RQNKFCEDDEMIYVGDE-TRDITAAQKSQVQ-VVAVAWGFNSPQILTQ---FNPDHLIHHPELLELDILD-----
 RQKQFKPQSIVYVGDE-TRDIEASKANIK-VIAVTWGFNSPEILAK---QNPDFLIHQPRELLEVIK-----
 RELGVKEPHTLMIQGDA-QGDIITMAKGAAQGAIAHWPGYAQGN---L---VGTDAITADLQQI-----
 EKLGVTFPAATLWVGDS-AGDIQMGKNAAGCIGICWGNAQVSY---L---ENADVAIAASLDEIK-----
 QALGTAVDKTLWVGDT-RADWEMAKQAKSAAAIAISWQPETHQD---L---QLADVVIRELTAIS-----
 QTLGVEPEATLWVGDA-VGDMQMARNAQAAGCIGITWV-NKPNN---V---QGANVVINQLDEI-----
 DKFGIJKSQICMVGDRLDIDILFGQNGCK-TLLVLSGIT-----
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 KKFGITTSQICMVGDRLDIDILFGQNGCK-TLLVLSGVTLSQTLQSFDSNISQPDFYTNQISDFLTL-----
 ERMGIAPOQMLFVGDS-RNDIQAAKAACGP-SVGLTYGYNYGEAIDL---SQPDFVIYQSINDLLPAGL-----
 NLLDVDTAQGVLVGDS-ADVAAARAAGCIP-VCLVRYGYAGPGGPAA---LGADALLDSLEALPALLTP-----
 A---ALTEEVVLYVGDS-EVDAATAEAAGLR-FAFTEGYRHAPVHDL---PHHGLFSHHDELDDL-----

430

.....|.....|.
Synechocystis_PCC_6714 -----**AIAT**
Synechocystis_PCC_6803 -----**AIAL**
Synechocystis_PCC_6803 -----**HICVE**
Microcoleus_FGP-2 -----**GHKN**
Microcystis_PCC_9701 -----**SIRAI****G**
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-RLA****PAA**
Rhodobacter_ATCC_17025 -----**HLLA**