

Supplementary Information

To the publication 'Gram-scale production of 4-vinyl guaiacol in the fast-growing cyanobacterium
Synechococcus sp. PCC 11901'

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1. Supplementary figures and tables

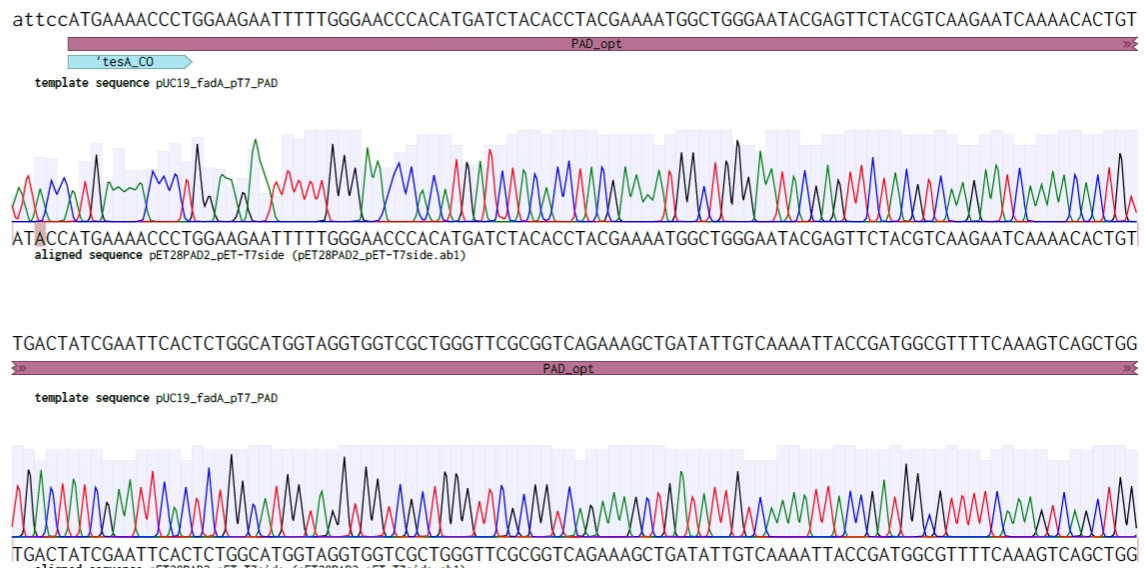


Figure 1 Sequence alignment of PAD with the amplicon from the colony-PCR of *S. PCC11901 fadA::pad*.

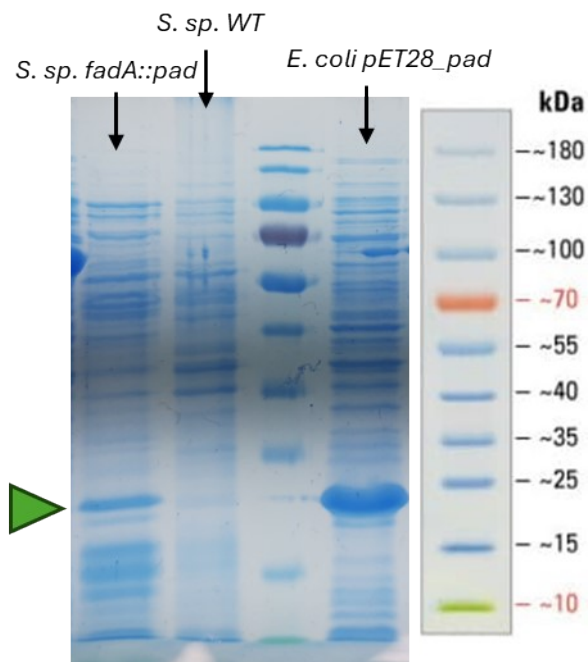


Figure 2 SDS-PAGE for expression control. Samples were denatured with an SDS-buffer at 95°C for 10min and transferred into a polyacrylamide gel (12% bis-acrylamide) and run at 200V. The marker is shown in the middle (PageRuler, Fisher Scientific, Waltham, MA, USA). *Synechococcus sp. PCC11901*, with left *fadA::pad* strain, after induction with 1mM IPTG, the middle lane is the WT of *S. PCC11901* followed by the DNA marker, outer right is an *E. coli* strain expressing PAD as a positive control. The molecular weight of PAD is expected at ~19 kDa, indicated by a green arrow.

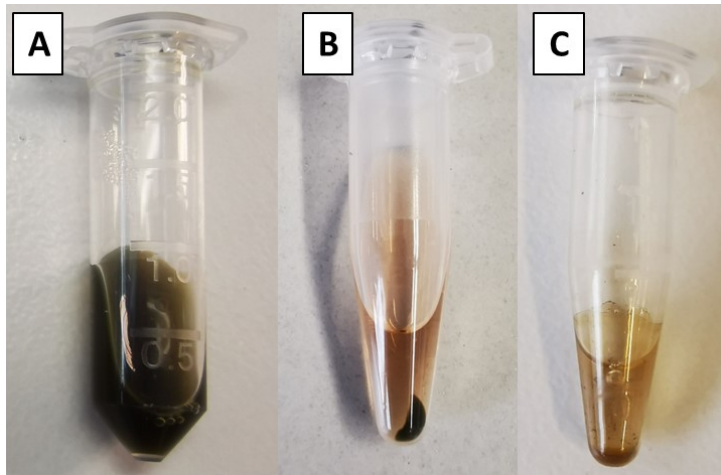


Figure 3 *Synechococcus* sp. PCC11901 *fadA::pad* was incubated with 10mM caffeic acid at 500 μ mol photons/s/m². **A** shows cells after 24h, **B** the same tube, after centrifugation. **C** shows 10mM 3,4-dimethoxy styrene after 24h incubation in the same buffer and light conditions, without cells.

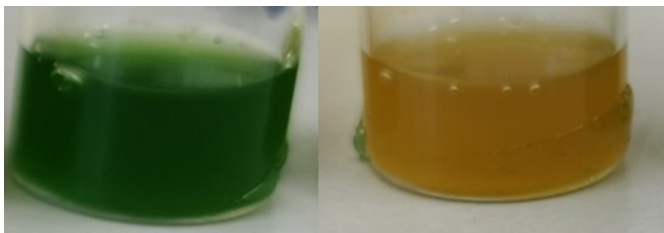


Figure 4 *Synechococcus* sp. PCC 11901WT (**left**) and *fadA::pad* (**right**) incubated with 10mM ferulic acid at 500 μ mol photons/s/m² after 24h.

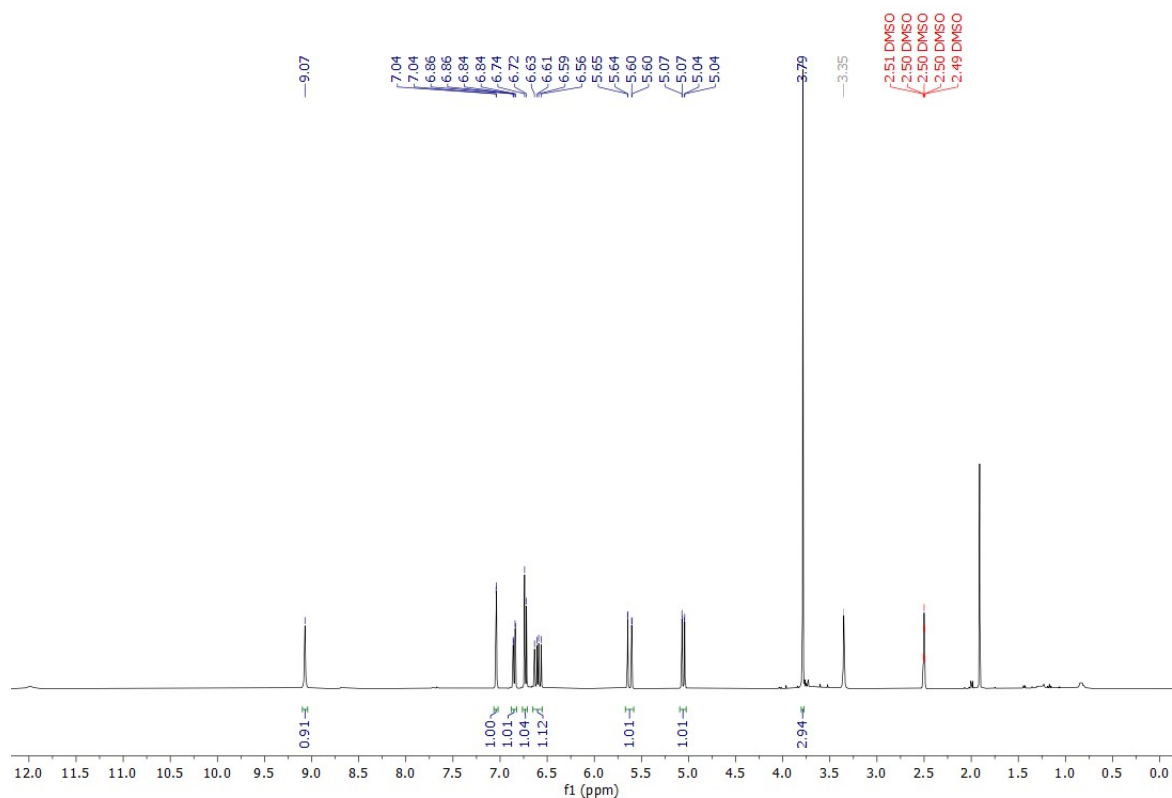


Figure 5 NMR spectrum of 4-vinyl guaiacol, converted from ferulic acid by *S. PCC 11901 fadA::pad*. The product was extracted from the organic phase (DINP) by basic extraction. NMR code: ^1H NMR (400 MHz, DMSO) δ 9.07 (s, 1H), 7.04 (d, J = 2.0 Hz, 1H), 6.85 (dd, J = 8.2, 2.0 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 6.60 (dd, J = 17.6, 10.9 Hz, 1H), 5.62 (dd, J = 17.6, 1.1 Hz, 1H), 5.06 (dd, J = 10.9, 1.1 Hz, 1H), 3.79 (s, 3H).

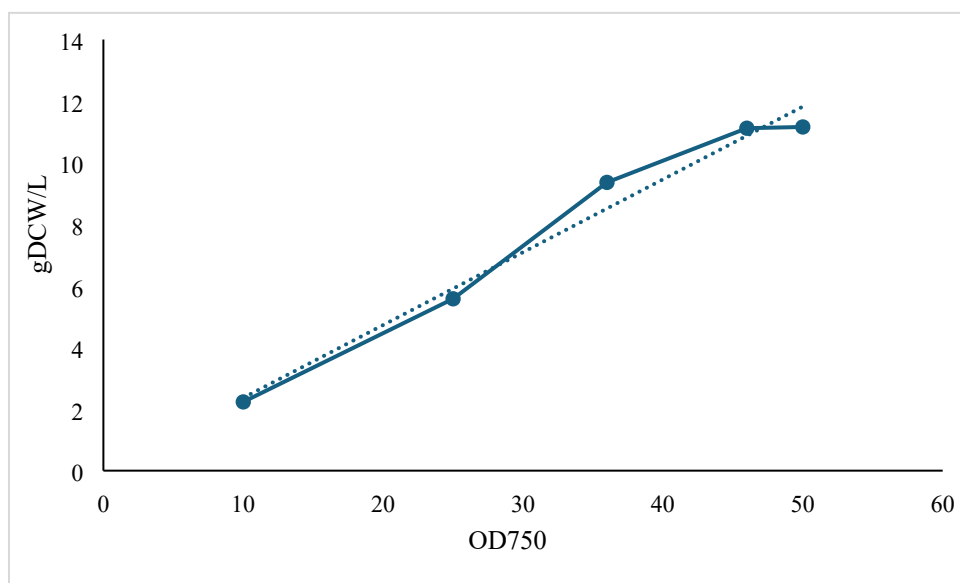


Figure 6 Calculating the factor of $\text{OD}_{750\text{nm}}$ to dry cell weight.

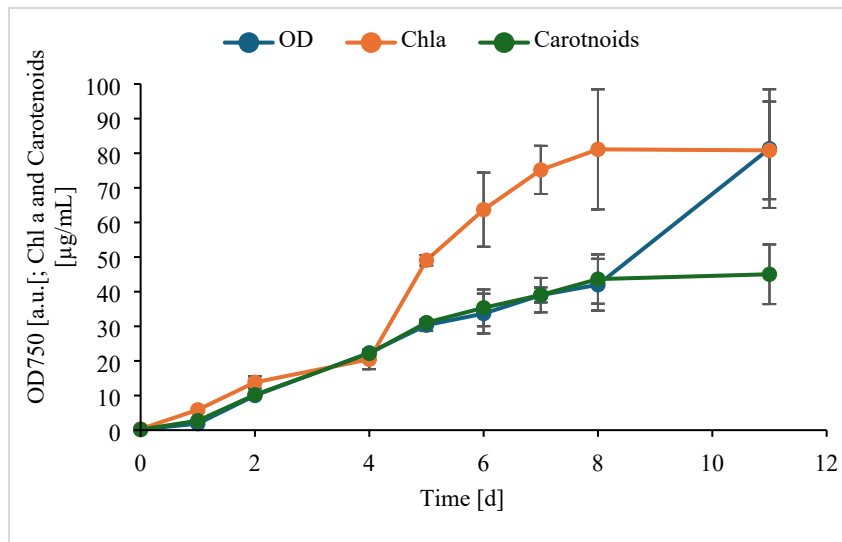


Figure 7 Optical density at 750nm (OD), Chlorophyll a and carotenoids content of *S. PCC 11901*, grown at 37°C, 1% CO₂, and 500 $\mu\text{mol photons/s/m}^2$. The error bars indicate the standard error of the means from 3 replicates.

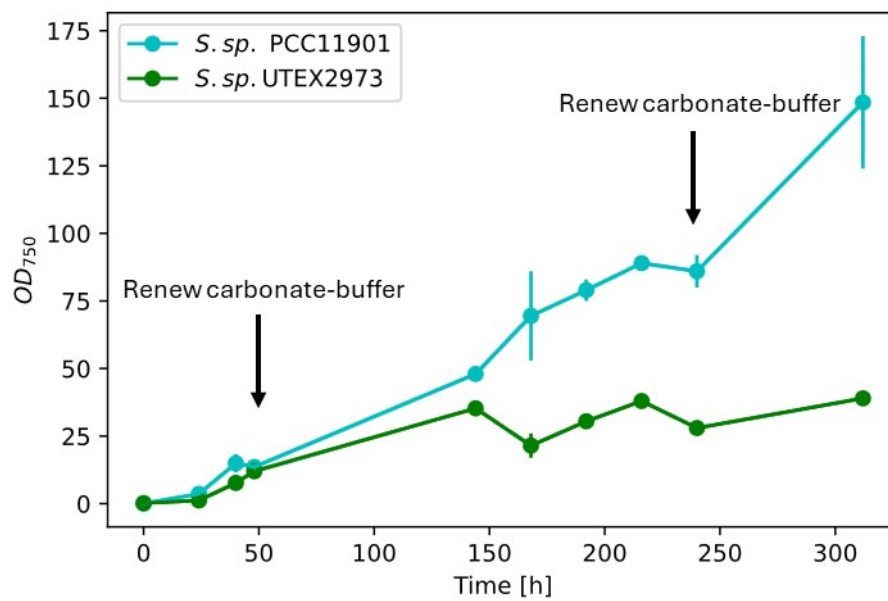


Figure 8 Growth comparison of *S. PCC11901* and *S. UTEX2973* in the CellDEG system. Cells were incubated at 500 $\mu\text{mol photons/s/m}^2$ and 37°C. The arrows indicate an exchange of the carbonate buffer. *S. UTEX2973* has been described as the fastest-growing cyanobacterium known¹, but *S. PCC11901* shows a higher total biomass accumulation.

Table 1 Media composition

Compound	LB	TB	AD7 ²	MAD2 ²	YBG11 ³
Peptone	10 g/L				
Yeast extract	5 g/L	24 g/L			
NaCl	5 g/L		18 g/L	18 g/L	
Tryptone		20 g/L			
KH ₂ PO ₄		17 mM	50 mg/L	50 mg/L	
K ₂ HPO ₄		72 mM		0.27605 g/L	0.0305 g/L
MgSO ₄ * 7H ₂ O			5 g/L	5 g/L	0.074 g/L
KCl			0.6 g/L	0.6 g/L	
NaNO ₃			1 g/L	16 g/L	1.49 g/L
CaCl ₂ * 2H ₂ O			0.37 g/L	0.37 g/L	36 mg/L
Na ₂ EDTA*2H ₂ O			30 mg/L	30 mg/L	5.95 mg/L
FeCl ₃ * 6H ₂ O			15 µM	30 µM	0.97 mg/L
Vitamin B12			4 µg/L	12 µg/L	
H ₃ BO ₃			2.86 µg/L	2.86 µg/L	2.78 mg/L
MnCl ₂ * 4H ₂ O			1.18 µg/L	1.18 µg/L	1.13 mg/L
ZnSO ₄ * 7H ₂ O			222 µg/L	222 µg/L	0.2 mg/L
Na ₂ MoO ₄ * 2H ₂ O			1.26 mg/L	1.26 mg/L	0.39 mg/L
CuSO ₄ *5H ₂ O			79 µg/L	79 µg/L	0.07 mg/L
CoCl ₂ * 6H ₂ O			40.3 µg/L	40.3 µg/L	0.16 µM
Citric acid * H ₂ O					6.3 mg/L
Na ₂ CO ₃					160 µM
Tris (pH 8.4)			1.0375 g/L	1.0375 g/L	
HEPES (pH 7.8)					10/100 mM (depends on use)
Bacto-Agar	18 g/L (for plates)		12 g/L (for plates)		
Na ₂ S ₂ O ₃			1 g/L (for plates)		
					Adjust to pH 7.2

2. Plasmids, primers, and sequences

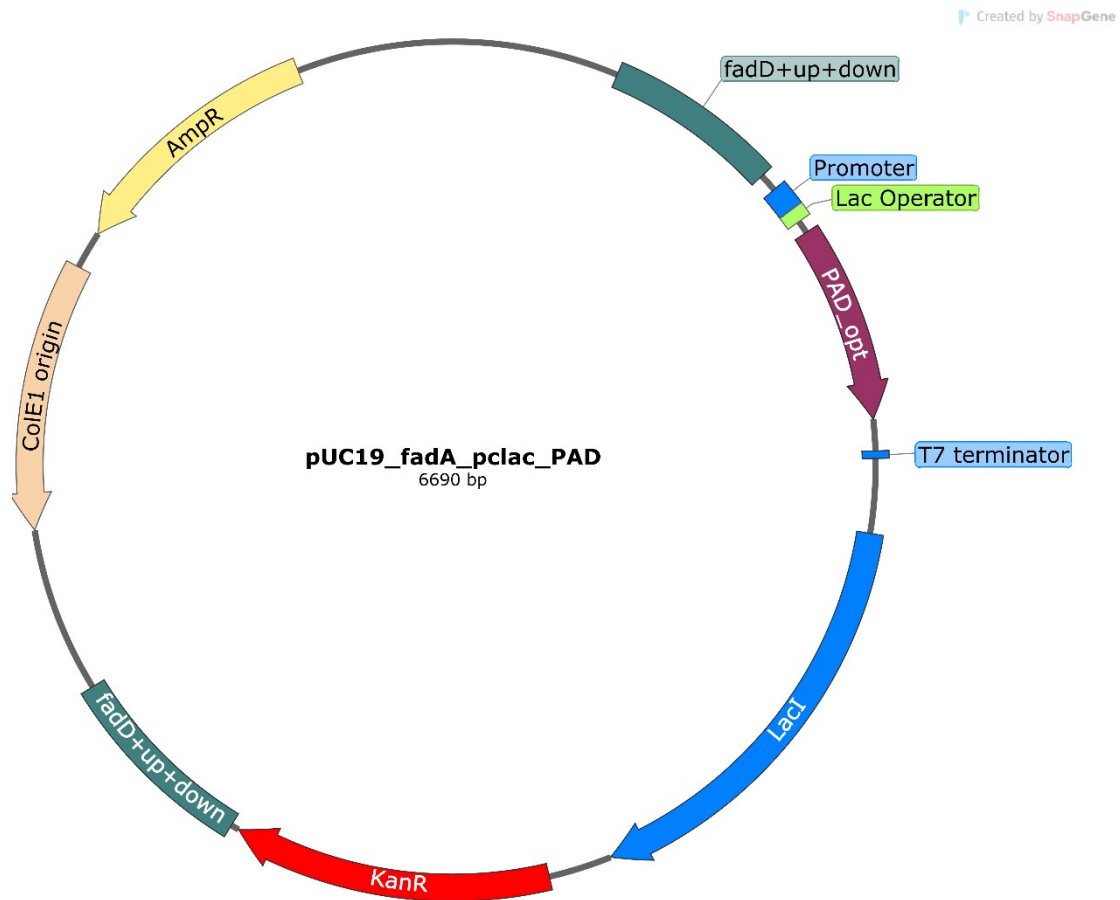


Figure 9 Plasmid for genomic integration of PAD into *S. PCC 11901*. The integration site was the *fadA* locus, as described in Włodarczyk et al., 2020.² The map was designed using SnapGene (Boston, USA).

2.1 Sequence of Phenolic Acid Decarboxylase (PAD) from *Bacillus coagulans* DSM11⁴

ATGAAAACCCTGGAAGAATTTTTGGGAACCCACATGATCTACACCTACGAAAATGGCTGGGAATACGAGTTCT
ACGTCAAGAATCAAAACACTGTTGACTATCGAATTCACCTCTGGCATGGTAGGTGGTCGCTGGGTTTCGCGGTCA
GAAAGCTGATATTGTCAAAATTACCGATGGCGTTTTCAAAGTCAGCTGGACGGAGCCGACAGGGACGGATGTC
AGTCTAAACTTCATGCCTGACGACAAGCGGATGCATGGGGTGATTTTTTTCCCGAAGTGGGTGCATGAGCATCC
CGAGATTACAGTCTGCTATCAGAATGATCACATCGATCTGATGGAAGAGTCGCGCGAAAAATATGAAACCTAT
CCCAAGTATGTGGTGCCAGAGTTTGCCGATATCACTTACATCAAAAATGAAGGCATCAACAACGAAAAAGGTGA
TCAGCGAGGCACCCCTACGCCACGATGGCGGATGACATTCGTTTCAGGCAAACCTCAAGTTTTCCTAA

2.2 Primers used in this study

Table 2 List of primers used in this study.

#74 PAD into fadA_fwd	tcaagtaggagattaattccATGAAAACCCTGGAAGAATTTTTGGG
#75 PAD into fadA_rev	gtttgtacaagaaatctagaTTAGGAAAACCTGAGTTTGCCTGAACG
#76 fadA for PAD_fwd	GCAAACCTCAAGTTTTCTAAAtctagatttctgtacaaactcggccc
#77 fadA for PAD_rev	AATTCTTCCAGGGTTTTCATggaattaatctcctacttgactttatgagt

3. References

1. Yu, J. *et al.* *Synechococcus elongatus* UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and CO₂. *Sci. Rep.*
2. Włodarczyk, A., Selão, T. T., Norling, B. & Nixon, P. J. Unprecedented biomass and fatty acid production by the newly discovered cyanobacterium *Synechococcus* sp. PCC 11901. *bioRxiv* (2020) doi:<https://doi.org/10.1101/684944>.
3. Shcolnick, S., Shaked, Y. & Keren, N. A role for mrgA, a DPS family protein, in the internal transport of Fe in the cyanobacterium *Synechocystis* sp. PCC6803. *Biochim. Biophys. Acta BBA - Bioenerg.* **1767**, 814–819 (2007).
4. Ni, J., Gao, Y.-Y., Tao, F., Liu, H.-Y. & Xu, P. Temperature-Directed Biocatalysis for the Sustainable Production of Aromatic Aldehydes or Alcohols. *Angew. Chem.* **130**, 1228–1231 (2018).