

Supplementary Tables for the paper entitled:

Early-branching cyanobacteria grow faster and up-regulate superoxide dismutase activity under a simulated early Earth anoxic atmosphere.

Supplementary Table 1: Primer sequences. Primers specific for the superoxide dismutase genes, *sodA* (MnSOD), *sodB* (FeSOD) and *sodC* (Cu/ZnSOD), identified in *Pseudanabaena* sp. PCC7367 were designed as indicated below, as well as the reference gene primer pair targeting the *rpoC1* gene encoding the RNA polymerase beta subunit (Enzingmüller-Bleyl et al., 2022).

Gene of interest	Region in genome CP003592.1	Corresponding protein (NCBI Accession)	Locus	Primer direction	Sequence (5' → 3')	Tm [°C]	GC content (%)	Product length in base pairs (bp)
<i>sodA</i>	770,159 to 770,920	WP_015163874.1	Pse7367_0596	Forward	GCGCTCATGCCTGCTAAA TC	60.04	55	167
				Reverse	CCTGATGACCCGTTTACG CT	60.11	55	
<i>sodB</i>	3,547,029 to 3,547,598	WP_015166022.1	Pse7367_2813	Forward	ACTGGCGCTTTGGCTAGT AA	57.3	50	165
				Reverse	GGGATTTTCAGCGTTGCC AG	60.11	56.4	
<i>sodC</i>	505,866 to 506,558	WP_015163683.1	Pse7367_0398	Forward	GCTGCTATGGGAGTTGTG GT	60.04	55	198
				Reverse	TTGGAGGTGATCGTTGAG GC	60.04	55	
				809R	GCTTCGGCACGGCTCG GGTCGATA	69,5	66,7	
<i>rpoC1</i>	n/a	n/a	Pse7367_0455	Forward	TGTTGGGTAAACGGGTTG AC	57.3	50	136
				Reverse	CGAATCAGGCGATTAATC ACAAA	57.31	39.1	

Supplementary Table 2: Primer binding efficiencies. The binding efficiency of the primers designed to detect the SOD genes were determined for both genomic DNA (gDNA) and copy DNA (cDNA) reverse transcribed from RNA extracted from *Pseudanabaena* sp. PCC7367.

Genes targeted for analysis are the *sodA* (MnSOD), *sodB* (FeSOD) and *sodC* (Cu/ZnSOD) genes with the *rpoC1* gene as reference gene (Enzingmüller-Bleyl et al., 2022). The primer efficiencies were determined using genomic DNA of *Pseudanabaena* sp. PCC 7367 ranging from 10 000 000 copies to 1 copy of the targeted gene, as well as dilutions of cDNA. All genes targeted were present in a single copy on the genome of *Pseudanabaena* sp. PCC 7367.

Gene of Interest	gDNA Dilutions	cDNA Dilutions	Primer Efficiency % with gDNA	Primer Efficiency % with cDNA
<i>rpoC1</i>	10 ng	10 ng	107.70	102.68
<i>sodA</i>	10 ng	10 ng	95.79	88.7
<i>sodB</i>	10 ng	10 ng	89.30	88.7
<i>sodC</i>	10 ng	10 ng	98.02	97.88

Supplementary Table 3: Secretory pathways encoded in *Pseudanabaena* sp. PCC7367. The presence of genes encoding putative *sec* (Russo & Zedler, 2021; Supplementary Tables S2 & S3) and *tat* (Russo & Zedler, 2021) (Supplementary Table S4) pathways were obtained through a similarity search using the characterised protein sequences obtained from *E. coli* K12 MI1665 or *Synechocystis* sp. PCC6803.

<i>Gene name</i>	Bait organism	Bait gene	<i>Pseudanabaena</i> PCC 7367 uniprotkb	BLASTp E value
<i>secA</i>	<i>E. coli</i> K12 MI1665	P10408 SecA	K9SFH1	6.81e-63
<i>secD</i>	<i>E. coli</i> K12 MI1665	P0AG90 SecD	K9SFP1	1.34e-61
<i>secF</i>	<i>E. coli</i> K12 MI1665	P0AG93 SecF	K9SFP1	1.81e-06
<i>secF</i>	<i>E. coli</i> K12 MI1665	P0AG93 SecF	K9SEI8	2.52e-31
<i>secY</i>	<i>E. coli</i> K12 MI1665	P0AGA2 SecY	K9SHY3	1.44e-107
<i>secE</i>	<i>Synechocystis</i> sp. PCC6803	P38382 SecE	K9SDF3	1.46e-16
<i>secG</i>	<i>Synechocystis</i> sp. PCC6803	P74508 SecG	K9SFY9	5.96e-21
<i>tatA</i>	<i>E. coli</i> K12 MI1665	P69428 TatA	K9SKB5	2.24e-05
<i>tatA</i>	<i>E. coli</i> K12 MI1665	P69428 TatA	K9SI06	5.16e-05
<i>tatC</i>	<i>E. coli</i> K12 MI1665	P69423 TatC	K9SCK0	2.55e-27

Supplementary Table 4: Metal transporters in *Pseudanabaena* sp. PCC7367. The presence of metal transporters encoded by *Synechocystis* PCC6803 for the metal cofactors required for the SOD isoforms investigated in this study, is indicated in the table below (modified from Sharon *et al.*, 2014). The characterized protein sequences from *Synechocystis* sp. PCC6803 (NC_000911.1) were used to search for similarity homologues in the genome of *Pseudanabaena* sp. PCC7367 (NC_019701.1) using tblastn (Altschul, 1991).

Metal	Gene name	Description	<i>Synechocystis</i> 6803 NC_000911.1 Gene locus tag	<i>Synechocystis</i> 6803 Protein ID	<i>Pseudanabaena</i> PCC 7367 NC_019701.1 Gene locus tag	Description	BLASTp E value
Mn	<i>mntC</i>	PsaA, metal ABC transporter substrate binding domain	SGL_RS08820	WP_010872546.1	WP_015163932.1	zinc ABC transporter substrate-binding protein	4e-44
	<i>mntA</i>	Inorganic Mn ²⁺ /Zn ²⁺ ion transporter ATPase component	SGL_RS08815	WP_010872545.1	WP_015163931.1	metal ABC transporter ATP-binding protein	8e-59
	<i>mntB</i>	ABC 3 transporter permease	SGL_RS0881	WP_010872544.1	WP_015166116.1	ABC-type Mn ²⁺ /Zn ²⁺ transport system, permease	1e-59
Zn	<i>znuB</i>	ABC-type Mn ²⁺ /Zn ²⁺ transport system, ATP-binding protein	SGL_RS04625	WP_010871740.1	WP_015163931.1	ABC-type Mn ²⁺ /Zn ²⁺ transport system, ATPase	1e-43
	<i>znuC</i>	hypothetical	SGL_RS04630	WP_010871742.1	/		No hit
	<i>znuB (ZntC?)</i>	zinc ABC transporter substrate-binding protein	SGL_RS04620	WP_010871739.1	WP_015166551.1	zinc ABC transporter substrate-binding protein	3e-28
Cu	<i>ctaA</i>	putative Cu binding site & ATP binding site	SGL_RS12475	WP_010873238.1	WP_051038173.1	copper-translocating P-type ATPase	0.0
	<i>pacS</i>	copper-translocating P-type ATPase	SGL_RS05435	WP_010871897.1	WP_015164254.1	copper-translocating P-type ATPase	0.0

Supplementary Table 5: Potential genes involved in the reduction of H₂O₂, the product of SOD dismutation of O₂^{•-}.

The presence of genes potentially encoding peroxidases or peroxiredoxins were obtained from the KEGG database (Kanehisa et al., 2022) for the genome of *Pseudanabaena* sp. PCC7367 (NC_019701.1). The presence of a glutathione synthase and glutathione reductase were also confirmed.

Description	<i>Pseudanabaena</i> sp. PCC7367 KEGG entry	Description	BLAST against the protein data bank and manual sequence inspection
Peroxiredoxin	Pse7367_1682	thioredoxin-dependent peroxiredoxin	BCP-type enzyme (alternative reductants)
Peroxiredoxin	Pse7367_2013	thioredoxin-dependent peroxiredoxin	Prx1-type enzyme (thioredoxin as a reductant)
Peroxiredoxin	Pse7367_2293	thioredoxin-dependent peroxiredoxin	BCP-type enzyme (alternative reductants)
Redoxin domain protein	Pse7367_2374	thioredoxin-dependent peroxiredoxin	Prx5-type enzyme (alternative reductants)
1-Cys peroxiredoxin	Pse7367_3020	thioredoxin-dependent peroxiredoxin	Prx6-type enzyme (reductant unknown)
Oxidoreductases	Pse7367_2328	peroxidase	Heme-containing peroxidase
Glutathione peroxidase	Pse7367_3393	glutathione peroxidase	Trx-dependent GPx (thioredoxin as a reductant)
Glutathione synthase	Pse7367_3280		
Glutathione reductase	Pse7367_0547	NADPH-glutathione reductase	

Supplementary Table 6: Results of One-way repeated measure ANOVA and/or ANOVA on ranks with environmental treatment condition as factor and Post-Hoc all pairwise multiple comparison procedures using Turkey Test and/or Holm-Sidak method for biomass parameters (Fig. 2 Suppl Fig 1). Bold numbers indicate significant differences with minimum $p \leq 0.05$. Number of replicates = 3.

One-Way repeated measure biomass parameter						
	One-way repeated measure ANOVA			ANOVA on ranks		
	F	p	DF	Chi-square	p	Df
Chlorophyll a				22,00	≤ 0.001	2
Carotenoid	8,443	0,002	2			
Chl a: carotenoid ratio	1.642	0.219	2			
Protein			2	15.048	≤ 0.001	2
Glycogen	7,01	0,004	2			
Growth	214,102	≤ 0.001	2			
Post-Hoc pairwise multiple comparison						
	LC vs. Archean		LC vs. HC		HC vs. Archean	
	<i>q/t</i>	<i>p</i>	<i>q/t</i>	<i>p</i>	<i>q/t</i>	<i>p</i>
Chlorophyll a	6.351	<0.001	3.175	0.064	3.175	0.064
Carotenoide	5.804	0.001	3.15	0.087	2.655	0.167
Protein	4.715	0.002	1.109	0.713	3.606	0.029
Glycogen	5.103	0.004	1.328	0.622	3.776	0.035
Growth	18.930	<0.001	2.227	0.068	16.703	<0.001

Supplementary Table 7: Results of One-way repeated measure ANOVA with environmental treatment condition as factor for growth media oxygen concentration (Suppl. Fig.2). In case of significant differences, Post-Hoc analysis using Holm-Sidak method was applied to identify differing groups: Bold numbers indicate significant differences with minimum $p \leq 0.05$. Number of replicates = 3.

One-Way repeated measure for growth media oxygen concentration						
Time after onset of monitoring	One-way repeated measure ANOVA					
	F		<i>p</i>		DF	
2h	3.399		0.137		2	
14h	2.826		0.172		2	
18h	17.941		0.01		2	
22h	7.411		0.045		2	
19h stirred	125.449		<0.001		2	
Post-Hoc pairwise multiple comparison						
Time after onset of monitoring	LC vs. Archean		LC vs. HC		HC vs. Archean	
	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
2h	n.s.					
14h	n.s.					
18h	5.988	0.012	2.872	0.045	3.117	0.07
22h	5.401	0.04	2.102	0.388	3.299	0.161
19h stirred	15.575	<0.001	5.289	0.006	10.286	0.001

Supplementary Table 8 : Results of repeated measure two way ANOVA using environmental treatment condition (Arch, HC, LC) as well as sampling time (Fig. 3, A-D) as factor for SOD gene expression, SOD protein activity and dissolved O₂ in the growth media. In case of significant differences, pairwise multiple comparison procedure using Duncan's Method for treatment (A) and time (B) as factor was applied to identify the respective groups of parameters that differ significantly. Bold numbers indicate significant differences with minimum $p \leq 0.05$. Number of replicates = 3.

	Treatment		Time		Treatment x Time	
	<i>F-value</i>	<i>p</i>	<i>F-value</i>	<i>p</i>	<i>F-value</i>	<i>p</i>
qPCR <i>sodA</i> (MnSOD)	5,156	0,078	9,183	0,004	24,669	<0.001
qPCR <i>sodB</i> (FeSOD)	105,979	<0.001	61,534	<0.001	27,317	<0.001
qPCR <i>sodC</i> (CuZnSOD)	125,71	<0.001	21,583	<0.001	6,632	<0.001
qPRC <i>sodABC</i> Sum	102,919	<0.001	22,448	<0.001	4,189	0,007
SOD enzyme activity	85,075	<0.001	540,712	<0.001	158,534	<0.001
Dissolved O ₂	11,069	0,023	112,171	<0.001	3,167	0,024
Post-Hoc pairwise multiple comparison						
A) Factor Treatment						
	LC vs. Archean		LC vs. HC		HC vs. Archean	
	<i>q</i>	<i>p</i>	<i>q</i>	<i>p</i>	<i>q</i>	<i>p</i>
qPCR <i>sodA</i> (MnSOD)	4,534	0,035	2,486	0,154	2,048	0,221
qPCR <i>sodB</i> (FeSOD)	16,783	<0.001	1,938	0,243	18,721	<0.001
qPCR <i>sodC</i> (CuZnSOD)	22,423	<0.001	11,361	0,002	11,063	0,002
qPRC <i>sodABC</i> Sum	18,704	<0.001	2,543	0,147	16,162	<0.001
SOD enzyme activity	17,118	<0.001	2,603	0,14	14,514	<0.001
Dissolved O ₂	6,652	0,01	3,484	0,07	3,167	0,089
B) Factor Time						
	qPCR <i>sodA</i> (MnSOD)		qPCR <i>sodB</i> (FeSOD)		qPCR <i>sodC</i> (CuZnSOD)	
	<i>q</i>	<i>p</i>	<i>q</i>	<i>p</i>	<i>q</i>	<i>p</i>
14.000 vs. 18.000	0.0145	0.992	12.15	<0.001	0.187	0.898
14.000 vs. 19.000	4.041	0.025	19.701	<0.001	11.234	<0.001
14.000 vs. 2.000	0.0272	0.985	17.263	<0.001	4.438	0.019
14.000 vs. 22.000	4.521	0.015	16.668	<0.001	1.943	0.224

18.000 vs. 19.000	4.056	0.028	7.552	0.001	11.047	<0.001
18.000 vs. 2.000	0.0416	0.979	5.113	0.008	4.251	0.02
18.000 vs. 22.000	4.507	0.013	4.519	0.013	1.756	0.25
2.000 vs. 19.000	4.014	0.022	2.439	0.123	6.796	0.002
22.000 vs. 19.000	8.563	<0.001	3.033	0.074	9.291	<0.001
22.000 vs. 2.000	4.549	0.017	0.594	0.685	2.495	0.116
	qPRC <i>sodABC</i> Sum		SOD enzyme activity		Dissolved O₂	
	<i>q</i>	<i>p</i>	<i>q</i>	<i>p</i>	<i>q</i>	<i>p</i>
14.000 vs. 18.000	0.0145	0.992	12.15	<0.001	0.187	0.898
14.000 vs. 19.000	4.041	0.025	19.701	<0.001	11.234	<0.001
14.000 vs. 2.000	0.0272	0.985	17.263	<0.001	4.438	0.019
14.000 vs. 22.000	4.521	0.015	16.668	<0.001	1.943	0.224
18.000 vs. 19.000	4.056	0.028	7.552	0.001	11.047	<0.001
18.000 vs. 2.000	0.0416	0.979	5.113	0.008	4.251	0.02
18.000 vs. 22.000	4.507	0.013	4.519	0.013	1.756	0.25
2.000 vs. 19.000	4.014	0.022	2.439	0.123	6.796	0.002
22.000 vs. 19.000	8.563	<0.001	3.033	0.074	9.291	<0.001
22.000 vs. 2.000	4.549	0.017	0.594	0.685	2.495	0.116

Supplementary Table 9: Pearson's correlation of parameters monitored during 24 h sampling (Fig. 3). Bold numbers indicate significant differences with minimum $p \leq 0.05$. Abrev. r = correlation coefficient, n = number of samples. Number of replicates = 3.

		qPCR <i>sodA</i>	qPCR <i>sodB</i>	qPCR <i>sodC</i>	qPCR <i>sodABC</i> sum	SOD enzyme activity	medium O ₂
Time	r	0.0796	-0.0238	-0.00657	0.0209	-0.0844	-0.0417
	p	0.603	0.877	0.966	0.892	0.582	0.785
	n	45	45	45	45	45	45
qPCR <i>sodA</i>	r		-0.149	0.521	0.47	0.349	0.234
	p		0.327	0.000242	0.00114	0.0186	0.122
	n		45	45	45	45	45
qPCR <i>sodB</i>	r			0.382	0.797	0.071	0.651
	p			0.00958	5.71E-11	0.643	0.00000127
	n			45	45	45	45
qPCR <i>sodC</i>	r				0.725	0.0142	0.675
	p				1.79E-08	0.926	0.000000376
	n				45	45	45
qPCR <i>sodABC</i> sum	r					0.242	0.751
	p					0.11	2.81E-09
	n					45	45
SOD enzyme activity	r						0.202
	p						0.183
	n						45

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