

SUPPLEMENTARY INFORMATION TO PAPER:

## The origin of teratogenic retinoids in cyanobacteria

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**Table S1. ALDH - BLAST comparison.** Similarity comparison of cyanobacterial and human enzymes containing CD F1-2. Part A shows basic information about enzyme annotation and origin. Part B shows BLAST comparison of individual enzymes. Abbreviations: C – percentage (%) cover of compared sequences; E – E-value; S - percentage (%) similarity.

<b><u>A</u></b>	<b>Accession number</b>	<b>Species</b>	<b>Enzyme</b>	<b>Conserved Domain</b>
<b>ToB</b>	WP_038075203.1	<i>Tolypothrix_boutellei</i>	ALDH	F1-2
<b>CroCh</b>	WP_008272605.1	<i>Crocospaera_chwakensis</i>	ALDH	F1-2
<b>H-A1</b>	NP_000680.2	<i>Homo_sapiens</i>	ALDH A1	F1-2
<b>H-A2</b>	NP_003879.2	<i>Homo_sapiens</i>	ALDH A2	F1-2
<b>H-2</b>	NP_000681.2	<i>Homo_sapiens</i>	ALDH 2	F1-2

  

<b><u>B</u></b>	<b>ToB</b>	<b>CroCh</b>	<b>H-A1</b>	<b>H-A2</b>	<b>H-2</b>
<b>ToB</b>		C100%; E0.0; S82.7%	C98%; E0.0; S63.7%	C97%; E0.0; S62.8%	C98%; E0.0; S67.4%
<b>CroCh</b>	C100%; E0.0; S82.7%		C98%; E0.0; S64.8%	C98%; E0.0; S63.7%	C98%; E0.0; S68.9%
<b>H-A1</b>	C98%; E0.0; S63.7%	C98%; E0.0; S64.8%		C98%; E0.0; S73.2%	C100%; E0.0; S68.1%
<b>H-A2</b>	C97%; E0.0; S62.8%	C98%; E0.0; S63.7%	C98%; E0.0; S73.2%		C96%; E0.0; S67.3%
<b>H-2</b>	C98%; E0.0; S67.4%	C98%; E0.0; S68.9%	C100%; E0.0; S68.1%	C96%; E0.0; S67.3%	

**Table S2. Basic characteristics of analyzed cyanobacterial aldehyde dehydrogenases.** Only one example sequence of aldehyde dehydrogenase for each conserved domain is presented.

Accession number	Organism	Enzyme	Conserved domain	Product	Function
WP_042155384.1	Planktothrix agardhii	CoA-acylating methylmalonate-semialdehyde dehydrogenase	ALDH_F6_MMSDH	propionyl-CoA	Amino acid metabolism - catabolism
WP_067774485.1	Nostoc sp. NIES 3756	proline/L-glutamate gamma-semialdehyde dehydrogenase	Pro_dh + ALDH_PutA	glutamate	two step catabolism of proline to glutamate
WP_067771050.1	unclassified Nostoc	aldehyde dehydrogenase family protein	CAJ73105	uncharacterized	-
WP_067769491.1	Nostoc sp. NIES 3756	glutamate-5-semialdehyde dehydrogenase	ProA	L-glutamate 5-semialdehyde and phosphate	L-proline biosynthesis
WP_067769931.1	Nostoc sp. NIES 3757	aldehyde dehydrogenase family protein	F1-2	retinoic acid; tetrahydrofolate and CO <sub>2</sub> ; acetate	oxidation of retinal; 10-formyltetrahydrofolate; acetaldehyde
ARI84357.1	Microcystis aeruginosa PCC 7806SL	NAD-dependent succinate-semialdehyde dehydrogenase	ALDH_SSADH1_GabD1	succinate	oxidation of succinate semialdehyde to succinate in the degradation of glutamate and 4-aminobutyrate
OBQ44124.1	Aphanizomenon flos-aque WA102	aldehyde dehydrogenase	YwdH	uncharacterized	oxidation of both apocarotenals and alkanals into corresponding acids
		aldehyde dehydrogenase	CALDH	apocarotenoid acid >C <sub>25</sub>	oxidation of both apocarotenals and alkanals into corresponding acids
WP_096594581.1	Calothrix sp. NIES 2098	aldehyde dehydrogenase family protein	DDAldH	4,4'-diapolycopene-diacid	4,4'-diapolycopene-dialdehyde into 4,4'-diapolycopene-diacid

**Table S3. MS parameters for analyzed retinoid compounds;** MRM (multiple reaction monitoring); quantification ions marked (bold).

Compound	MRM transition	Cone voltage (V)	Collision energy (V)
4OH-ATRA	<b>299.2</b> → <b>95.2</b>	30	23
	299.2 → 157.2	30	25
4keto-ATRA	<b>315.2</b> → <b>137.1</b>	30	25
	315.2 → 241.2	30	15
4keto-RAL	<b>299.2</b> → <b>147.2</b>	30	24
	299.2 → 189.2	30	16
5,6epoxy-ATRA	<b>317.2</b> → <b>107.2</b>	30	21
	317.2 → 153.2	30	14
9/13 <i>cis</i> -RA	<b>301.2</b> → <b>159.1</b>	30	23
	301.2 → 205.1	30	13
ATRA	<b>301.2</b> → <b>159.1</b>	30	23
	301.2 → 205.1	30	13
RAL	<b>285.3</b> → <b>161.2</b>	30	9
	285.3 → 175.2	30	13
4keto-13 <i>cis</i> -RA	<b>315.3</b> → <b>137.1</b>	30	25
	315.3 → 241.2	30	15
4keto-9 <i>cis</i> -RA	<b>315.3</b> → <b>137.1</b>	30	25
	315.3 → 241.2	30	15
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ATRA-d5	<b>306.2</b> → <b>162.2</b>	30	20
	306.2 → 206.2	30	15
RAL-d5	<b>290.3</b> → <b>161.2</b>	30	8
	290.3 → 180.3	30	15
4keto-ATRA-d3	290.3 → 198.2	30	7
	<b>318.2</b> → <b>137.2</b>	30	24
4keto-13 <i>cis</i> -RA-d3	318.2 → 162.2	30	14
	318.2 → 244.2	30	14
	<b>321.2</b> → <b>143.1</b>	30	25
	321.2 → 247.2	30	15

**Table S4. Primers and PCR.** Sequences of used primers and PCR conditions for Illumina library preparation.

	Primer		Sequence	Product size	Reference
<b>V4 16S rRNA gene</b>	<b>16S_F</b>	forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-InnerTag-GTGYCAGCMGCCGCGGTAA	~ 300	<i>Caporaso et al., 2011; Apprill et al., 2015</i>
	<b>16S_R</b>	reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGC-InnerTag-GGACTACNVGGGTWTCTAAT		

<b>V4 16S rRNA gene</b>			
Thermal cycling conditions			
Temperature	Time	cycle	
95°C	30 s		
98°C	10 s		
55°C	15 s	30x	
72°C	30 s		
72°C	10 min		
10°C	10 min		

## **References:**

- Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, 75(2), 129–137. <https://doi.org/10.3354/ame01753>
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, 108(SUPPL. 1), 4516–4522. <https://doi.org/10.1073/pnas.1000080107>

List of other Supplementary materials (attached MS Excel files)

**Supplementary material S1**

Excel sheet S1a: Retinoids concentration in cultures of axenic cyanobacteria

Excel sheet S1b: Taxonomic composition of cultures of axenic cyanobacteria based on 16S data

**Supplementary material S2**

Excel sheet: Cyanobacterial ALDHs - sequences

**Supplementary material S3**

Excel sheet: Cyanobacterial 16S rRNA - sequences

**Supplementary material S4**

Excel sheet: Exp 1 Data – Concentration, REQchem

**Supplementary material S5**

Excel sheet: Exp 2 Data – Concentration, REQchem

**Supplementary material S6**

Excel sheet: Optimization of Fenton reaction