

Supplementary Information for the article in Journal of Applied Phycology:

Characterizing the role of bromide in the transcriptional regulation and expression of the cyanobacterial neurotoxin aetokthonotoxin.

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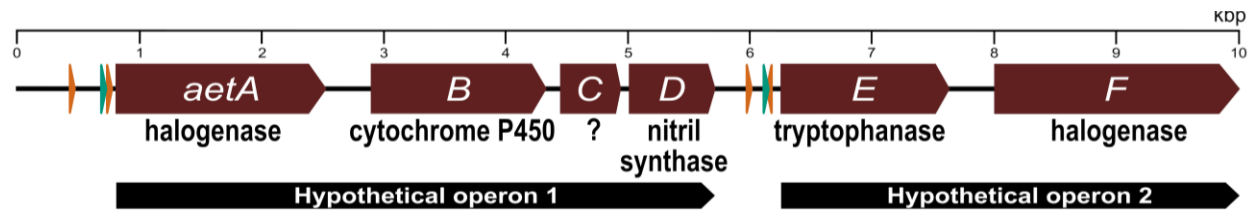
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a)



b)

IS4-Transposase GGT TTT GTCTATTGTTTCATTCTCTTTAGACTTCACCC
 TGAAACTCCACCCAACAGCACCCCTTATGAGATCAGCAAATAGCCATCACG
 CTCCCTGCGATGCTCATGCACACCGCCGAGATTGCGATCAGCGCTTCG
 CGATCACCCCAAAAACAACAGCGTTTCGATTCTCTCAATCCTGGATGACG
 AGGATTCTAATCCTTTAATCCCTTCTATGCACATTTATCTAATCCTTTAA
 TCCCTTCTATGCACATCCAGTTCAATCCCATTCTATCCCGTTCACTTCCT
 CCCCATAATCATTCACTCACCAATTTGTATACTTTTGTCTTTTGTGCGA
 AGCGAAGTCAGCCTCAAATCGTTACTTCATCGAGCTTCCACCTTTTGTGTC
 ACCCTAATTTTCTTCTTATAAGCTAATTGTTACTTCTGTTTGCAGTAGT
 TATTTAACTTTAC TTTTCTTCGTATTCTCTTGACTTTTCCAACTACTTC
 TGCTGCCTCGTTCTCCGAAAATCGCTGTTTGTGCTTCCACTGTTTTGACT
 ACAACTCCCGCAGCATAACCCTTTTTCGCCTTTCCTTACTTTGTTACGAA
 ACATGTGACTAAAGCATAGCCTCACAAAATCGCGTTGCTCGCCGAAAGCC
 TTAAGTTGAAATTAACCTTGCCAAAGTTGCCTGGTGTCAGCTAGTTAAAAT
 ATGCAAACGTGATTTGTATAAGAGCTACAGAGTTATTTGCTAAATAACTC
 TTAGTGTTGACCAGTTATGCTGCTAATGAGCAACCAGTAACCTGAGATATCG
 CGAATTAGAGCTACTGTTATTAAAAAGTAAACACGGTAAAACTCATAT
 GTATGATGTTGTGATTTGTGGGGNGGGGGCTGGCTGGTCTCAGTTTAGCG
 AGACAATTAAG Start of *aetA*

c)

End of *aetA* GGTGAAGTGATTAAAGCAATGCGAGTTTCGACAAATAT
 AAAAAACCCCTCTCCAAACCTCTCCCTGCGAGCGGCGTCTGTGCCAACT
 TGTAGCAAGTGGCTTTAAAGGCTAAATTTTCGTTGGTAATCAAGAGACAT
 CTCCGAAAATGAATCTAGAACCTTTGTGTCAGCTTGGGCTGATACCTAATT
 TCTGGAGATGTCTTGTCTAAGGATATTTCTGACCCTTTCAGCGTCGGAGA
 GAGGTTGGGCAAAGGTTCAACGAATTCACCTTTAAAGCAATCACCTTGGTA
 AAAACTCGGATAACCGCTACAACATCCTTGTTAACCTTTTTTCTAGCCAA
 CCTAGGAGATACTC Start of *aetB*

d)

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End of aetD GCAATACAATTTCAGATAAGCCCCAAAACCCTGATTTAG
AGTTAGAGACGCGATTTATCCCGTCTTCAAAGACCAATTATCTACGCCAA
TAACCCTTAACTGAACAGTTAGGGGATTTCACGAGTTTTTTAGCCGGCGAT
GGAGCTAGGATTACCTATCGTAGGATCGAGTTACCCCGAGAAGACATCTT
CGATGGACTTAAACAGCAGTTTCGTCTATGTCTATACGTCGGATGACATAG
ACAACACGCAGCTTTTCACTCGAAGCTCCCCTGGCGTTGTGGTCTGGAT
TATAACGACGATGGCGATGAAGTCTTTTTCTCAACGAGATCAGCGTCTT
TCTACATGGGTCTGGGGACAGTTTCCATTGACGAGCGAGTACGGTTCGTT
GTTTAGTCCTGTAAGTAGCAAGTGTACAATAACTTACGCTAAGGCTGTAA
TCAAATCACAGCAATGGTTTCAGAAGCTATGATTTGTAAAGTGTCGTCTA
AAAAAGAACAACAATATTTGAAGGTAGAAG Start of aetE

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e)

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End of aetE AGACTTCTATGTTTCCAGATTAAACCAAACTTCCGAG
AATTGAACAAGACAGTCCCGATGAAAATAATTTTCACCGCCAGGCATGTAA
GAGGAATATTCCTACTCCCTCTTACAAGTCAGAAGGCGGGAGGCAGAGG
GCAGAAAGTTCCGTACATTTCGGCGATTCAACGCCACTTGAACCGGAGCCA
CCAAATTTCCAATTTGGTGGGGGTCTTAAACCCTTGGTCTTTTCAGTCGC
GCAGAGGGTTCGGGTATTCAGAGAAGGGGATGAATCTCTTCTGCCCTCTA
CCTTTCCTTGAAAATTCTTCTTAATAGATGAGTAAGTGAGGTAAAACCTA
Start of aetF

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Figure S1. *In silico* analysis of NtcA (■) and NdhR (■) binding sites upstream and within the *aet* gene cluster, indicating a complete (■) or partial (■) TNNNNT box. a) Graphical representation of OTUs in the *aet* cluster and predicted binding sites. b-d) Intergenic regions containing the predicted binding sites.

a)

◀ End of *fabI* ACAAATCAGCTTTAATGCAATCGATCCTTAAAAGGAT
ACTAGAATCTGAGCCTAATTTGACTTTGTTTTTGGGAGGATTTATAAATA
ATGATTATTAACAAGCATGTTTACGACCATGAACTACTGCTTATTTCTT
AAGATATTCTCAAGATGTCCTTCTTTTGTCTTCATAAACTTTTGGTTA
CAACCGTTCAAGCAATTATAAAAAAGTAGTAAATTTAAGAATGAAAAAA
CTTGTATCACAATGAACAAATAATTATGATAAAAAGGGGTTAAGTATAGG
AAAGTACAAAAAGGTTGACAATGTCCTTATIGATTTTAAAGGTGTATTCTT
AGGTAACTCTATTTTAGTTTTCCGGCATTAGGTAGGGGAAGGGAG ▶ *ntcA*

b)

◀ End of *ccmK* ATGCTCATAGGGCATAGGACATGGGGCATTGGGAAAA
TAAAGTATGTAATTAAATTTGTCTCTATGAACCTTATAAACAAAGCAGATT
TTAGGAAGGATGGTAGATGCGGGAAATTTAAAAAAACGTTATTCACACCT
AGCAGTATAAGTAACATATTCTAAGGTGTGAAACGTTGAGAGCAAGGAGG
AAGGTGTCTTACGCTTGTGTTCCCTTTCATACTAACTTGCAAAACCTATAA
GAGAAATACTAAATTTATAATAAAGATAATAGATGTTTATCTATAGTTTT
TTGTTTTACAAACA CTGTGAGGGGATAACCC ▶ *ndhR*

Figure S2. *In silico* analysis of NtcA (■) and NdhR (■) binding sites upstream of *ntcA* and *ndhR*, indicating a complete (■) or partial (■) TNNNNT box.

Table S1: Primer pairs designed for this work.

Gene	Function	Purpose	Primer name	Sequence
<i>aetF</i>	Tryptophan halogenase	<i>aet</i> transcription reporter	aetFF	TAGTAAGTCGTCGCCTCC
			aetFR	GCCACATCGCTATATCATTC
<i>prsA</i>	Kinase in the pentose phosphate pathway	normalization	prsAF	GCGGAACATACTACTTACAGAG
			prsAR	ACAATTCCAATTCCAGAGAGC
<i>rimM</i>	16S rRNA processing protein	normalization	rimMF	CCTCACCATCAACTTACAACC
			rimMR	CTAGCAGGACGTTATATTGAAGG
<i>secA</i>	Part of the Sec protein translocase complex	normalization	secAF	GTGTTAAGCAGGAGTACGAAC
			secAR	GTAAC TGGTTGTCAATTCGCC

Table S2: Two way ANOVA for normalized AETX content on short-term time series.

Source of Variation	% of total variation	SS	D F	MS	F (DFn, DFd)	P value
Interaction	29.53	4751782589485	8	593972823686	F (8, 30) = 30.9 6	P<0.000 1
Time	21.13	3400610783770	4	850152695942	F (4, 30) = 44.3 2	P<0.000 1
Treatments	45.76	7362363172901	2	368118158645	F (2, 30) = 191. 9	P<0.000 1
Residual	-	575506605326	3 0	19183553511		

Table S3: Dunnett's multiple comparisons test for normalized AETX levels of each treatment vs the AETX levels on the Zero bromine samples at their corresponding sampling point for the short-time series.

Comparison	Mean Diff.	95.00% CI of diff.	Below Low?	Summary	Adjusted P Value
Inoculation					
Against Low	-157806	-420270 to 104657	No	ns	0.2905
Against High	-148525	-410989 to 113938	No	ns	0.3300
0.66 hrs					
Against Low	-316397	-578861 to -53934	Yes	*	0.0167
Against High	-181389	-443852 to 81074	No	ns	0.2053
2 hrs					
Against Low	-444875	-707338 to -182411	Yes	***	0.0009
Against High	-1342210	-1604673 to -1079747	Yes	****	<0.0001
4 hrs					
Against Low	-486716	-749180 to -224253	Yes	***	0.0003
Against High	-2050975	-2313438 to -1788512	Yes	****	<0.0001
8 hrs					
Against Low	-192979	-455442 to 69484	No	ns	0.1711
Against High	-1136934	-1399398 to -874471	Yes	****	<0.0001

Table S4: Two way ANOVA for normalized AETX content on long-term time series.

Source of variation	%	SS	DF	MS	F	P value
Interaction	23.59	4.148e+014	6	69132006124580	F (6, 24) = 39.75	P<0.0001
Time	18.88	3.321e+014	3	1.107e+014	F (3, 24) = 63.65	P<0.0001
Treatments	55.16	9.701e+014	2	4.850e+014	F (2, 24) = 278.9	P<0.0001
Residual		41743384648779	24	1739307693699		

Table S5: Dunnett's multiple comparisons test for normalized AETX levels of each treatment vs the AETX levels on the control samples at their corresponding sampling point for the long-time series.

Comparisons	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Inoculation					
Against Low	-157806	-2687818 to 2372206	No	ns	0.9844
Against High	-148525	-2678537 to 2381487	No	ns	0.9861
1 day					
Against Low	-696645	-3226657 to 1833367	No	ns	0.7449
Against High	-9704732	-12234743 to -7174720	Yes	****	<0.0001
3 days					
Against Low	-2480376	-5010387 to 49636	No	ns	0.0551
Against High	-16779226	-19309238 to -14249214	Yes	****	<0.0001
6 days					
Against Low	-3720235	-6250247 to -1190224	Yes	**	0.0039
Against High	-20515846	-23045858 to -17985834	Yes	****	<0.0001

Table S6: Two way ANOVA for normalized $\log_2$ *aet* expression on short-term time series.

Source of variation	% variation	SS	DF	MS	F (DFn, DFd)	P value
Interaction	20.46	9.527	8	1.191	F (8, 30) = 1.762	P=0.1248
Time	20.92	9.741	4	2.435	F (4, 30) = 3.602	P=0.0163
Treatment	15.07	7.02	2	3.51	F (2, 30) = 5.192	P=0.0116
Residual		20.28	30	0.676		

Table S7: Dunnett's multiple comparisons test for normalized $\log_2$ *aet* transcription levels of each treatment vs the $\log_2$ *aet* levels on the control samples at their corresponding sampling point on short-term time series.

Comparisons	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
At inoculation					
Against Low	-0.7770	-2.335 to 0.7810	No	ns	0.4141
Against High	-1.063	-2.621 to 0.4950	No	ns	0.2127
0.6 hours					
Against Low	-0.5280	-2.086 to 1.030	No	ns	0.6511
Against High	-1.687	-3.245 to -0.1290	Yes	*	0.0326
2 hours					
Against Low	-1.633	-3.191 to -0.07499	Yes	*	0.0391
Against High	-0.2760	-1.834 to 1.282	No	ns	0.8850
4 hours					
Against Low	-1.418	-2.976 to 0.1400	No	ns	0.0778
Against High	0.1060	-1.452 to 1.664	No	ns	0.9819
8 hours					
Against Low	-0.1380	-1.696 to 1.420	No	ns	0.9695
Against High	-0.8770	-2.435 to 0.6810	No	ns	0.3335

Table S8: Dunnett's multiple comparisons test for normalized $\log_2$ *aetF* transcription levels of each treatment/sampling time vs the levels on the control samples at the start of the experiment for short term samples.

Comparisons	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Against Zero bromine samples					
at 0.6 hr	-1.331	-3.343 to 0.6811	No	ns	0.3641
at 2 hr	-0.839	-2.851 to 1.173	No	ns	0.8548
at 4 hr	-1.055	-3.067 to 0.9571	No	ns	0.639
at 8 hr	-1.026	-3.038 to 0.9861	No	ns	0.6703
Against Low bromine samples					
at inoculation	-0.777	-2.789 to 1.235	No	ns	0.9023
at 0.6 hr	-1.859	-3.871 to 0.1531	No	ns	0.0827
at 2 hr	-2.472	-4.484 to -0.4599	Yes	**	0.0096
at 4 hr	-2.473	-4.485 to -0.4609	Yes	**	0.0095
at 8 hr	-1.164	-3.176 to 0.8481	No	ns	0.5232
Against High bromine samples					
at inoculation	-1.063	-3.075 to 0.9491	No	ns	0.6304
at 0.6 hr	-3.018	-5.030 to -1.006	Yes	**	0.0011
at 2 hr	-1.115	-3.127 to 0.8971	No	ns	0.5747
at 4 hr	-0.949	-2.961 to 1.063	No	ns	0.7513
at 8 hr	-1.903	-3.915 to 0.1091	No	ns	0.0718

Table S9: Two way ANOVA for normalized $\log_2$ *aet* expression on long-term time series.

Source of variation	% variation	SS	DF	MS	F (DFn, DFd)	P value
Interaction	13.21	5.302	6	0.8836	F (6, 24) = 1.973	P=0.1096
Time	58.66	23.55	3	7.851	F (3, 24) = 17.53	P<0.0001
Treatment	1.356	0.5443	2	0.2721	F (2, 24) = 0.6076	P=0.5528
Residual		10.75	24	0.4479		

Table S10: Dunnett's multiple comparisons test for normalized $\log_2$ *aet* transcription levels of each treatment vs the $\log_2$ *aet* levels on the control samples at their corresponding sampling point on long-term time series.

Comparisons	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
At inoculation					
Against Low	-0.7770	-2.061 to 0.5068	No	ns	0.2818
Against High	-1.063	-2.347 to 0.2208	No	ns	0.1127
1 day					
Against Low	0.9950	-0.2888 to 2.279	No	ns	0.1424
Against High	0.5490	-0.7348 to 1.833	No	ns	0.5089
3 days					
Against Low	0.6820	-0.6018 to 1.966	No	ns	0.3667
Against High	1.279	-0.004843 to 2.563	No	ns	0.0509
6 days					
Against Low	0.2260	-1.058 to 1.510	No	ns	0.8839
Against High	0.1690	-1.115 to 1.453	No	ns	0.9328

Table S11: Dunnett's multiple comparisons test for normalized $\log_2$ *aetF* transcription levels of each treatment/sampling time vs the levels on the control samples at the start of the experiment for long-term samples.

Comparisons	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Against zero bromine samples					
at 1 day	-2.954	-4.576 to -1.332	Yes	***	0.0001
at 3 days	-2.694	-4.316 to -1.072	Yes	***	0.0005
at 6 days	-2.845	-4.467 to -1.223	Yes	***	0.0002
Against low bromine samples					
at inoculation	-0.777	-2.399 to 0.8447	No	ns	0.6888
at 1 day	-1.959	-3.581 to -0.3373	Yes	*	0.0124
at 3 days	-2.012	-3.634 to -0.3903	Yes	**	0.0098
at 6 days	-2.619	-4.241 to -0.9973	Yes	***	0.0007
Against high bromine samples					
at inoculation	-1.063	-2.685 to 0.5587	No	ns	0.3471
at 1 day	-2.405	-4.027 to -0.7833	Yes	**	0.0017
at 3 days	-1.415	-3.037 to 0.2067	No	ns	0.1096
at 6 days	-2.676	-4.298 to -1.054	Yes	***	0.0005

Table S12: Identification of LTTRs (LysR-Type Transcriptional Regulator) homologues in *A. hydriilicola* Thurmond2011 with NdhR-binding sites according to Prodoric.

Consensus LysR (COG0583) hits against <i>A. hydriilicola</i> Thurmond2011			Conserved Protein Domain		Closest hits on the PDB database			Score to NdhR-binding sites (MX000406)		
Accession	Size (AA)	% Identity	Accession	Name	Accession	Name	% Identity	Matrix Score	Core Score	Relative score
WP_208348325.1	302	30.34	cd08419	CbbR-like LTTR involved in the CO ₂ fixation	5Y2V_A	NdhR (Regulator of Ci assimilation)	67.5	7.85	6.99	0.96
WP_208339935.1	314	43.02	COG0583	LysR-like transcriptional regulator	8H3V_S	NtcB (Nitrogen assimilation co-regulator)	81.7	7.85	6.99	0.91
WP_208351416.1	302	33.91	cd08422	LysR-type transcriptional regulator CrgA	3MZ1_A	Putative LTTR in <i>iSinorhizobium meliloti</i> 1021 with crystal structure	31.7	8.09	6.99	0.94
WP_208354676.1	296	31.54	cd08414	LTTRs involved in the catabolism of aromatic compounds	3K1M_A	BenM (Transcriptional regulator for benzoate degradation)	34.5	8.29	6.99	0.96

Table S13: Identification of NtcA homologue in *A. hydriilicola* Thurmond2011 with NtcA-binding sites according to ProDoric.

Consensus NtcA(TIGR03697) hits against <i>A. hydriilicola</i> Thurmond2011			Conserved Protein Domain		Closest hits on the PDB database			Score to NtcA-binding sites (MX000209)		
Accession	Size (AA)	% Identity	Accession	Name	Accession	Name	% Identity	Matrix Score	Core Score	Relative score
WP_015141263.1	223	83	COG0664	Regulatory subunit of cAMP-dependent protein kinases	5Y2V_A	NtcA Global nitrogen regulator	99.5	10.04	8.5	0.96

Table S14: Identification of binding sites for NtcA and NdhR in the *aet* gene cluster with relevant Prodoric confidence scores.

Intergenic region	BP before Transcription start site	Prodoric ID	Organism	Matrix Score	Core Score	Relative score
Pre- <i>aetE</i>	-229	NdhR – MX000406:	<i>Synechocystis sp.</i> (Strain PCC 6803)	8.41	6.85	0.97
	-94	NtcA – MX000209	<i>Synechocystis sp.</i> (Strain PCC 6803)	8.62	7.17	0.92
	-74	NdhR – MX000406:	<i>Synechocystis sp.</i> (Strain PCC 6803)	8.16	6.99	0.94
Pre- <i>aetA</i>	-358	NdhR – MX000406:	<i>Synechocystis sp.</i> (Strain PCC 6803)	8.35	6.85	0.97
	-43	NtcA – MX000210	<i>Synechococcus sp.</i> (Strain PCC 7002)	8.07	6.42	0.87
	-15	NdhR – MX000406:	<i>Synechocystis sp.</i> (Strain PCC 6803)	7.66	6.85	0.89