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Article

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Light-independent phytoplankton degradation and detoxification of methylmercury in water

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SUPPLEMENTARY INFORMATION

Supplementary Table 1. Previous studies and observations related to methylmercury (MeHg) degradation by phytoplankton

Publication year	Phytoplankton species	Cell density (cells/mL)	MeHg conc. (pM)	MeHg/Cell ratio (amol/cells)	Incub. period (h)	Incubation conditions	Demethyl. rate constants	Potential drivers, mechanisms	Reference
2007	Field communities	Not provided	0.5–1	-	24	Diurnal, 24-h dark, Non-axenic	Diurnal: 0.003–0.01 h^{-1} Dark: 0–0.0045 h^{-1}	Photochemical, Dark: microbial	Monperrus et al. [1]
2007	Field communities	Not provided	0.15 – 250	-	0-55	Diurnal, Dark, Non-axenic	0.004–0.18 h^{-1}	Photochemical, dark microbial	Whalin et al. [2]
2014	Field communities	Not provided	1	-	24	Diurnal, 24-h dark, Non-axenic	Diurnal: 0.0–0.02 h^{-1} Dark: 0.003–0.009 h^{-1}	Mainly photochemical	Sharif et al. [3]
2021	Field communities	$(1.1\text{-}2.8) \times 10^6$	100	0.036-0.091	24	Diurnal, Non-axenic	0.002-0.005 h^{-1}	Photochemical, dark microbial	Cossart et al. [4]
2014	*Chlamydomonas reinhardtii*	1.0×10^5	0.7–99	0.007-0.77	48	Light, Axenic	0.0036 h^{-1}	Photochemical, microbial	Bravo et al. [5]
2018	*Isochrysis galbana*	5.6×10^5	3.8×10^4	67.86	240	Visible light, Axenic	0.001 h^{-1}	Likely photochemical	Kritee et al. [6]
2019	Field bacterioplankton	5.3×10^6	40	0.0075	96	Diurnal; Non-axenic	6.12×10^{-4} h^{-1}	Microbial, bacterioplankton	Lee & Fisher [7]
2022	*15 Marine microalgae*	$\sim1.0 \times 10^5$	10	0.1	96	Light Non-axenic	0.002-0.008 h^{-1}	Photochemical, dark microbial	Li et al. [8]
2016 2017 2021	*Chlamydomonas reinhardtii*	$\sim1 \times 10^6$	10 – 5×10^4	0.01-50	2	Light Axenic	Not observed	No demethylation (unknown)	Beauvais-Fluck et al.[9] [10] Slaveykova et al. [11]

Supplementary Table 2. Estimated cysteine (CYS) thiolate contents from translated genome sequences of *Chlorella vulgaris* UTEX 395, *Synechocystis sp.* PCC6803 and *Microcystis sp.* 0824.

Algal species	Number of genes	Total amino acid residues	Total CYS	CYS as % of total amino acids
***Chlorella vulgaris* CV395**	7112	2942860	54367	1.85
***Synechocystis sp.* PCC6803**	3507	1122720	11388	1.01
***Microcystis sp.* 0824**	4164	1111521	11305	1.02

Supplementary Table 3. Homologs of key *mer* operon genes present in algal strains used in this study. *Chaetoceros gracilis* was excluded from the analysis since no complete genomes are currently available. E-values and alignment scores are reported in **Supplementary Table 4**.

	merA	***merB***	***merR***	***merT***	***merD***
***Chlorella vulgaris* CV395**	+	-	-	-	-
***Synechocystis sp.* PCC6803**	+	-	+	-	-
***Microcystis sp.* 0824**	+	-	+	-	-

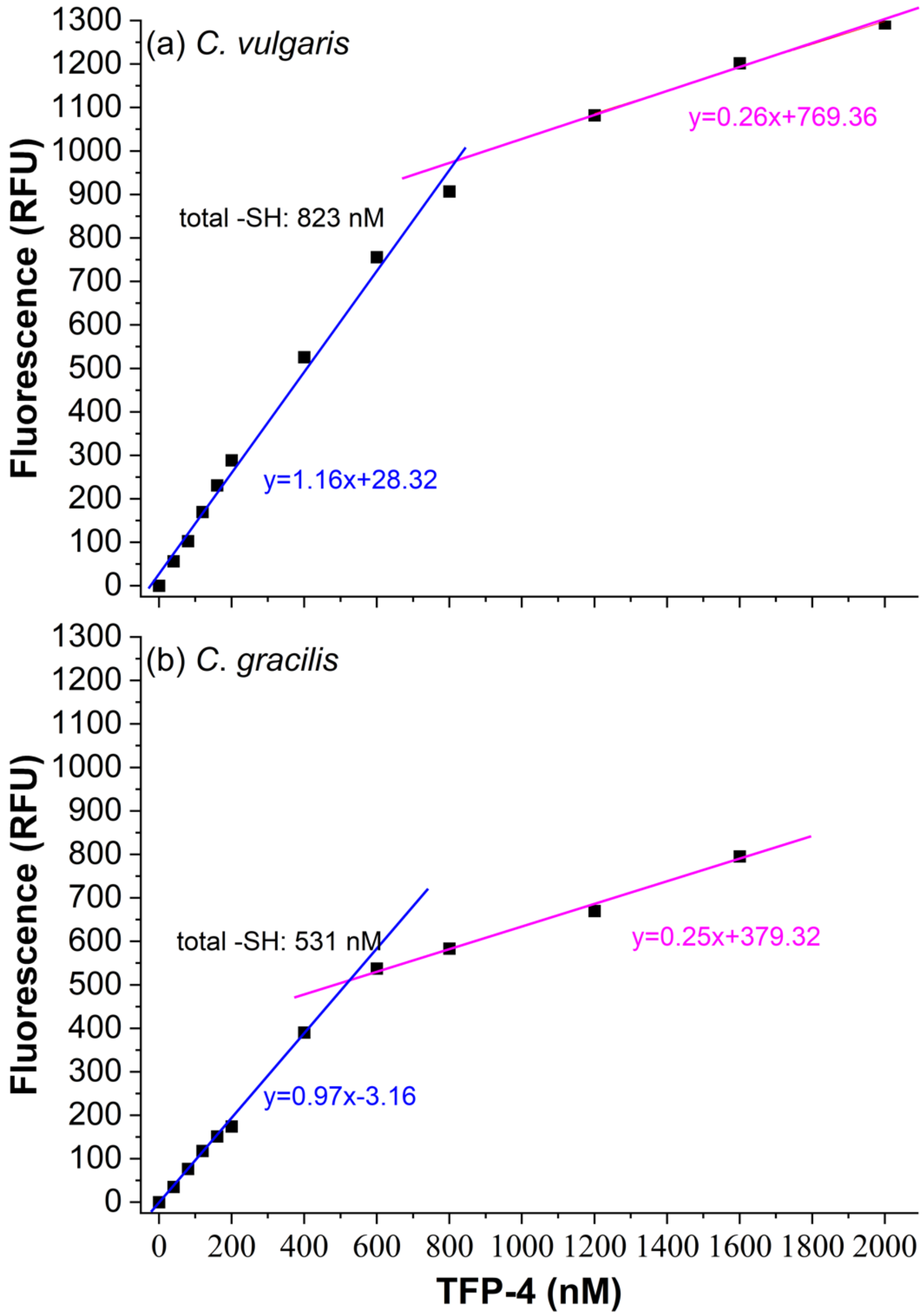
Supplementary Table 4. BLASTP alignment scores for homologs identified using the translated sequences of *merA and merR* from the pDU1358 *mer* operon as a template.

Strain	Query gene	Homolog ID	E-value	Identity [%]	Score	Coverage [%]
***Chlorella vulgaris* CV395**	*merA*	Scaffold_1889	$7 \cdot 10^{-53}$	30	186	87
	merR	-	-	-	-	-
***Synechocystis sp.* PCC6803**	*merA*	P73059	$1 \cdot 10^{-58}$	32	201	76
	merR	Q55963	$1 \cdot 10^{-14}$	31	67	80
***Microcystis sp.* 0824**	*merA*	A0A2L2XMF5	$4 \cdot 10^{-61}$	31	207	76
	merR	A0A2L2XI87	$3 \cdot 10^{-11}$	31	58	49

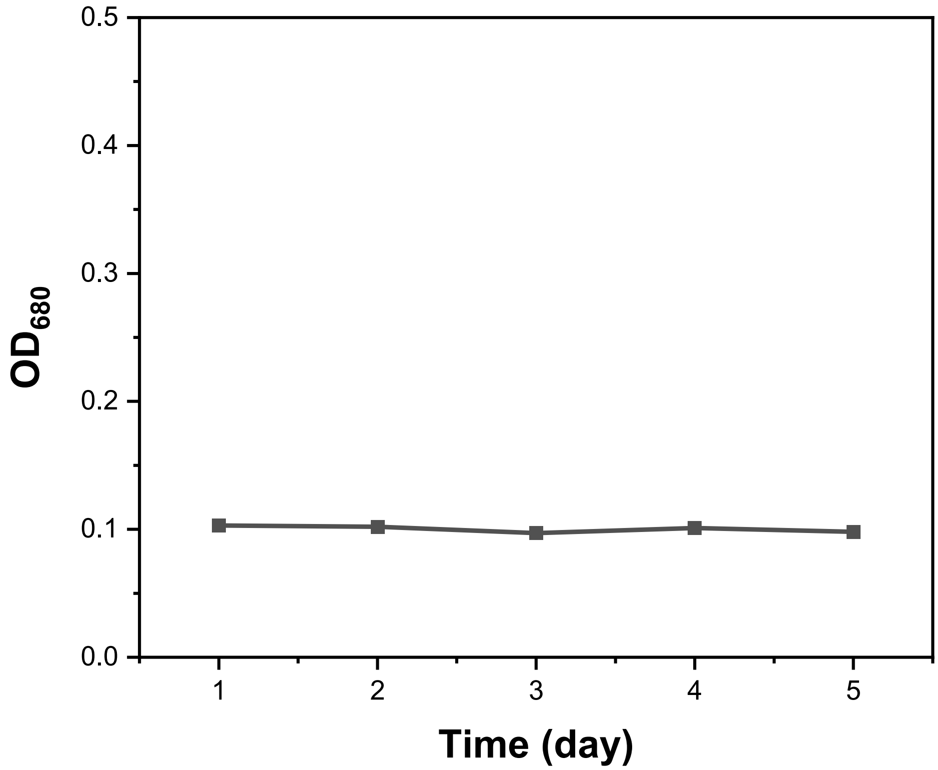
Supplementary Table 5. Aqueous chemical properties of the simulated freshwater (SFW), simulated seawater (SSW), filtered lake water and seawater. The seawater was collected from the coast of Venice Beach in California, United States. The lake waters were collected, respectively, from Melton Lake in Oak Ridge, Tennessee, United States and Yangshan Lake in Nanjing, Jiangsu Province, China.

Aqueous composition	SFW[a]	SSW[b]	Melton Lake water[c]	Seawater[c]	Yangshan Lake water
Cl^- (mg/L)	17.7	18610	2.8	17660	-
NO_3^- (mg/L)	0	0	2.6	9.5	-
Br^- (mg/L)	0	0	0	81.55	-
SO_4^{2-} (mg/L)	28.8	2590	18.2	2780	34.4
PO_4^{3-} (mg/L)	-	-	0	3.6	-
HCO_3^- (mg/L)	61	140	-	-	-
DOC (mg/L)	-	-	1.49	0.69	1.06
pH	8.00	7.90	7.98	8.03	7.39

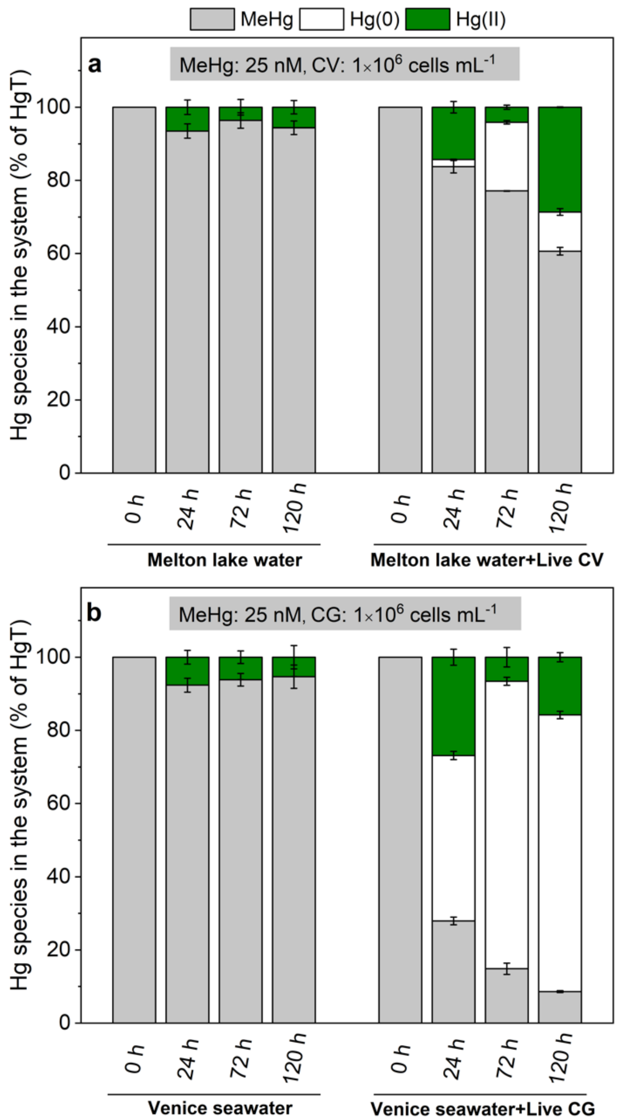
[a] SFW consists of 0.084 g L^{-1} $NaHCO_3$, 0.0292 g L^{-1} NaCl, and 0.0426 g L^{-1} Na_2SO_4 at pH 8.0 [12].
[b] SSW contains 26.5 g L^{-1} NaCl, 5.28 g L^{-1} $MgCl_2$ $6H_2O$, 6.65 g L^{-1} $MgSO_4$ $7H_2O$, 1.47 g L^{-1} $CaCl_2$ $2H_2O$, 0.72 g L^{-1} KCl, 0.20 g L^{-1} $NaHCO_3$ at pH = 7.9 [13].
[c] Major dissolved anions were analyzed by ion chromatography (Dionex DX-120, Sunnyville, CA, USA), and dissolved organic carbon (DOC) concentrations were determined using a Shimazu TOC-5000A total organic carbon analyzer, as previously described [14].



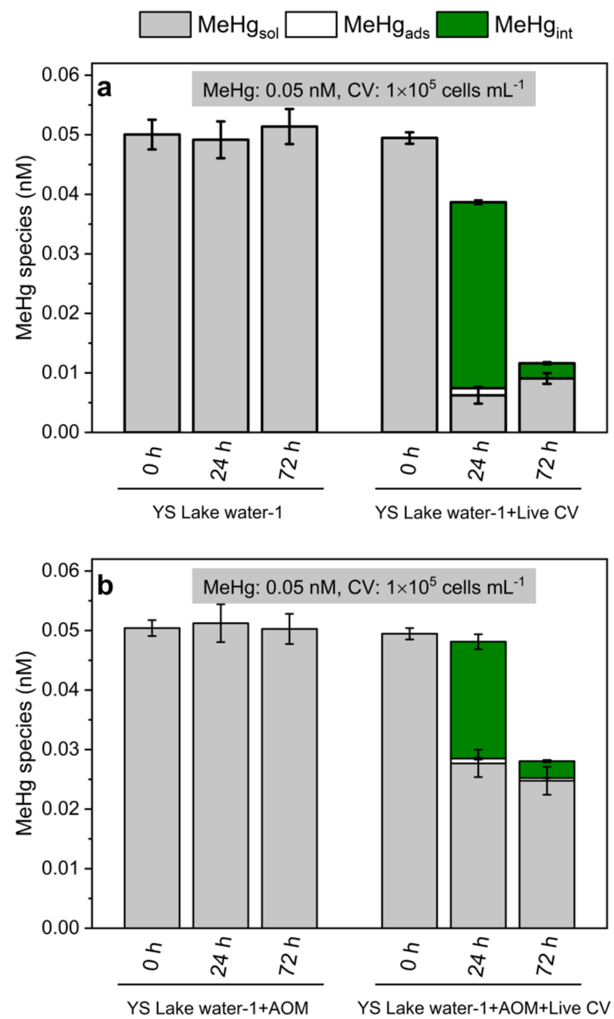
Supplementary Fig. 1. Determination of cell thiol contents in *Chlorella vulgaris* CV395 and *Chaetoceros gracilis* CG2568. Cell lysates of (a) CV395 (at 5×10^{6} cells mL^{-1}) and (b) CG2568 (at 6×10^{6} cells mL^{-1}) were reacted with the thiol fluorescent Probe IV (TFP-4) under anoxic conditions [15]. Fluorescence intensities were measured against TFP-4 concentrations and cell thiol contents determined using previously established methods [16, 17]. The estimated cellular thiol contents were about 165 amol·$cell^{-1}$ for CV395 and 88 amol·$cell^{-1}$ for CG2568.



Supplementary Fig. 2. The changes of OD values of the *Chlorella vulgaris* CV395 culture during incubation with 25 nM methylmercury (MeHg). The initial OD value was 0.1, measured at 680 nm.



Supplementary Fig. 3. Methylmercury (MeHg) and its degradation products [inorganic Hg(II) and Hg(0)] during dark incubation between MeHg (25 nM) and *Chlorella vulgaris* CV395 in Melton Lake water (a), and between MeHg and *Chaetoceros gracilis* CG2658 in Venice seawater (b) at 28 °C. Water samples were filter-sterilized by passing through 0.2-µm syringe filters, and the added cell concentration was 1×10^6 cells mL^{-1}. The measured Hg species was normalized to the total Hg (HgT) at each timepoint. Data are mean ± 1 SD (n = 2).



Supplementary Fig. 4. Concentrations and distributions of MeHg during degradation by *Chlorella vulgaris* CV2338 in Yangshan (YS) lake water-1 either in the absence (a) or presence (b) of algal organic matter (AOM). CV cells (1×10^5 cells mL^{-1}) were incubated with 0.05 nM MeHg at 28 °C. DOC concentrations in YS lake water-1 and AOM amended YS lake water-1 were 1.06 and 14.3 mg/L, respectively. Data are mean ± 1 SD (n = 2, 3, or 4).

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