

Electronic Supplementary Information

Arsenolipid biosynthesis by the unicellular alga *Dunaliella tertiolecta* is influenced by As/P ratio in culture experiments

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Table S1 Parameters for the quantification and speciation of arsenic species with HPLC-ICPMS/ESMS

HPLC	Agilent 1100 and/or Dionex Ultimate 3000
Arsenolipids	
Column	ZORBAX Eclipse XDB-C8 (4.6 x 150 mm, 5µm)
Column temperature	30 °C
Injection volume	40 µL (ICPMS) or 10 µL (ESMS)
Flow rate	0.8 mL min ⁻¹
Mobile phase	A: 0.1 % formic acid in water B: 0.1 % formic acid in EtOH
Gradient	0 min, 50 % B; 0-30 min, 100 % B; 30-35 min, 100 % B
Splitter	20 % delivered to ICPMS (ICPMS only)
Support flow	1 % formic acid in water containing Ge and Te as internal standards; delivered at 0.8 mL min ⁻¹ (ICPMS only)
Gradient compensation	20 % EtOH in water delivered with ISIS at 0.02 rpm (ICPMS only)
Arsenic cations	
Column	Agilent IonoSpher 5C (4.6 x 100 mm, 5 µm); or ZORBAX 300-SCX (4.6 x 150 mm, 5 µm)
Column temperature	30 °C
Injection volume	20 µL (ICPMS) or 10 µL (ESMS)
Flow rate	1 mL min ⁻¹
Mobile phase	10 mM pyridine, pH 2.6 (with IonoSpher 5C); or 20 mM ammonium formate including 3 % MeOH, pH 2.6 (SCX-300)
Arsenic anions	
Column	Hamilton PRP-X100 (4.6 x 150 mm, 5 µm)
Column temperature	30 °C
Injection volume	20 µL (ICPMS only)
Flow rate	1 mL min ⁻¹
Mobile phase	5 mM malonate, pH 5.6
ICPMS (arsenolipids)	Agilent 7500ce
Mode	Time resolved analysis
RF power	1550 W
Carrier gas	0.7 mL min ⁻¹
Optional gas	10 % O ₂
Masses recorded	m/z 53 (Cr), 74 (Ge), 75 (As), 77 (Se), 125 (Te)
ICPMS (arsenic ions)	Agilent 7900
Mode	Time resolved analysis
RF power	1550 W
Carrier gas	1.1 mL min ⁻¹
Optional gas	12 % CO ₂
Recorded masses	m/z 53 (Cr), 75 (As), 77 (Se)
HR-ESMS	Thermo Fisher – Q-Exactive Hybrid Quadrupole-Orbitrap
Mode	Positive and/or negative; ddMS ²
Spray voltage	3.4 kV
Capillary temperature	365 °C
S-lens RF level	50.0
Resolution	70 000 (FWHM)
Scan range	m/z 300-1100
NCE for ddMS ²	10-100

Table S2. HPLC-ICPMS data for As-species and concentrations ($\mu\text{g As g}^{-1}$ dry algae) in cultured *Dunaliella tertiolecta* (mean values \pm standard deviations of $n = 7$). Statistically significant differences from control (no added As/P) are depicted as * $p < 0.05$; ** $p < 0.01$; or *** $p < 0.001$. Concentrations of seven arsenolipids in CRM Hijiki previously reported ²⁹ are presented in parentheses (*italics*) for comparison.

species/regime	no added As or P	low As / low P	high As / low P	low As / high P	high As / high P	CRM Hijiki
DMA	0.14 \pm 0.06	0.35 \pm 0.13**	0.82 \pm 0.24***	0.20 \pm 0.06	0.47 \pm 0.13***	0.61 \pm 0.08
MA	0.03 \pm 0.01	0.04 \pm 0.01*	0.07 \pm 0.02***	0.01 \pm 0.01***	0.01 \pm 0.01**	0.01 \pm 0.01
AsSug482	0.14 \pm 0.03	0.44 \pm 0.09***	0.90 \pm 0.34***	0.83 \pm 0.21***	1.47 \pm 0.21***	1.11 \pm 0.22
As(V)	0.92 \pm 0.30	1.42 \pm 0.39*	2.64 \pm 1.22**	2.47 \pm 0.43***	6.20 \pm 2.71***	10.64 \pm 1.15
AB	0.04 \pm 0.02	0.02 \pm 0.01	0.06 \pm 0.03	0.01 \pm 0.01*	0.03 \pm 0.01	0.03 \pm 0.01
AsSug328	0.44 \pm 0.10	2.10 \pm 0.65***	3.85 \pm 0.96***	0.97 \pm 0.45*	1.86 \pm 0.63***	0.60 \pm 0.14
AsCatU	0.06 \pm 0.02	0.18 \pm 0.07***	0.38 \pm 0.15***	0.05 \pm 0.04	0.22 \pm 0.06***	< 0.01
AsMeSug268	0.13 \pm 0.04	0.09 \pm 0.02*	0.13 \pm 0.06	0.03 \pm 0.01***	0.02 \pm 0.01***	< 0.01
TMAO	< 0.01	0.01 \pm 0.01	0.04 \pm 0.02***	0.01 \pm 0.01	0.01 \pm 0.01	< 0.01
AsMeSug250	0.29 \pm 0.09	0.29 \pm 0.08	0.64 \pm 0.13***	0.21 \pm 0.08	0.23 \pm 0.08	< 0.01
AsLipU1-5	0.51 \pm 0.14	1.46 \pm 0.36***	1.90 \pm 0.25***	1.91 \pm 0.23***	2.44 \pm 1.03***	0.63 \pm 0.05
AsHC332	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	1.07 \pm 0.05 (1.07 \pm 0.04)
AsLipU6	0.12 \pm 0.04	0.12 \pm 0.03	0.12 \pm 0.02	0.09 \pm 0.02*	0.11 \pm 0.02	< 0.01
AsHC360	0.14 \pm 0.02	0.24 \pm 0.02***	0.30 \pm 0.05***	0.26 \pm 0.03***	0.27 \pm 0.05***	0.13 \pm 0.02 (0.09 \pm 0.01)
AsSugPhytol546	2.67 \pm 0.47	3.10 \pm 0.51	4.41 \pm 0.45***	2.54 \pm 0.46	2.52 \pm 0.59	< 0.01
AsSugPL978	0.21 \pm 0.10	0.51 \pm 0.12***	0.81 \pm 0.25***	1.38 \pm 0.29***	1.24 \pm 0.33***	< 0.01
AsSugPL958	0.15 \pm 0.02	0.31 \pm 0.06***	0.40 \pm 0.06***	0.88 \pm 0.10***	1.09 \pm 0.21***	2.41 \pm 0.12 (1.59 \pm 0.03)
AsSugPL986	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.34 \pm 0.02 (0.30 \pm 0.01)
AsSugPL1014	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.23 \pm 0.01 (0.21 \pm 0.01)
AsSugPL1042	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.15 \pm 0.01 (0.11 \pm 0.01)
AsLipU7	0.18 \pm 0.04	0.13 \pm 0.03*	0.21 \pm 0.04	0.04 \pm 0.01***	0.05 \pm 0.01***	< 0.01
AsSugPL1070	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.05 \pm 0.01 (0.04 \pm 0.01)

Table S3 Accurate masses of intact arsenolipids (bold type) and their fragments (normal type) in *D. tertiolecta* cultures measured by HPLC-HR-ESMS/MS. For AsSugPL956, AsSugPL982 and AsSugPL984 no MS/MS data could be obtained due to their low concentrations

Compound name	Formula/fragment [M+H] ⁺	Theoretical m/z [M+H] ⁺	Measured m/z [M+H] ⁺	Δm [ppm]
AsHC360	C₁₉H₂₄AsO	361.2446	361.2441	1.30
	C ₂ H ₈ OAs	122.9786	122.9786	0.33
	C ₂ H ₆ As	104.9680	104.9683	1.52
AsSugPhytol546	C₂₈H₅₆O₅As	547.3338	547.3336	0.37
	C ₈ H ₁₈ O ₅ As	269.0364	269.0363	0.82
	C ₈ H ₁₆ O ₄ As	251.0259	251.0258	0.59
	C ₅ H ₁₂ O ₃ As	194.9997	194.9997	0.19
	C ₂ H ₈ OAs	122.9786	122.9787	1.44
	C ₆ H ₇ O ₂	111.0441	111.0444	2.83
	C ₂ H ₆ As	104.9680	104.9684	3.92
AsSugPL956	C₄₅H₈₇AsO₁₄P	957.5043	957.5058	1.45
AsSugPL958	C₄₅H₈₉AsO₁₄P	959.5200	959.5199	0.19
	C ₁₀ H ₂₃ AsO ₁₀ P	409.0239	409.0239	0.17
	C ₁₀ H ₂₂ O ₇ As	329.0576	329.0577	0.42
	C ₇ H ₁₄ O ₄ As	237.0102	237.0102	0.13
	C ₅ H ₅ O ₂	97.0284	97.0286	2.06
AsSugPL978	C₄₇H₈₅AsO₁₄P	979.4887	979.4884	0.35
	C ₁₀ H ₂₃ AsO ₁₀ P	409.0239	409.0239	0.17
	C ₁₀ H ₂₂ O ₇ As	329.0576	329.0577	0.42
	C ₇ H ₁₄ O ₄ As	237.0102	237.0102	0.13
	C ₂ H ₈ OAs	122.9786	122.9788	1.62
	C ₂ H ₆ As	104.9680	104.9682	1.90
	C ₅ H ₅ O ₂	97.0284	97.0285	1.03
AsSugPL980	C₄₇H₈₇AsO₁₄P	981.5043	981.5046	0.19
	C ₁₀ H ₂₃ AsO ₁₀ P	409.0239	409.0239	0.17
	C ₁₀ H ₂₂ O ₇ As	329.0576	329.0577	0.43
	C ₇ H ₁₄ O ₄ As	237.0102	237.0102	0.12
	C ₅ H ₅ O ₂	97.0284	97.0286	1.55
AsSugPL982	C₄₇H₈₉AsO₁₄P	983.5200	983.5189	0.28
AsSugPL984	C₄₇H₉₁AsO₁₄P	985.5356	985.5362	0.51

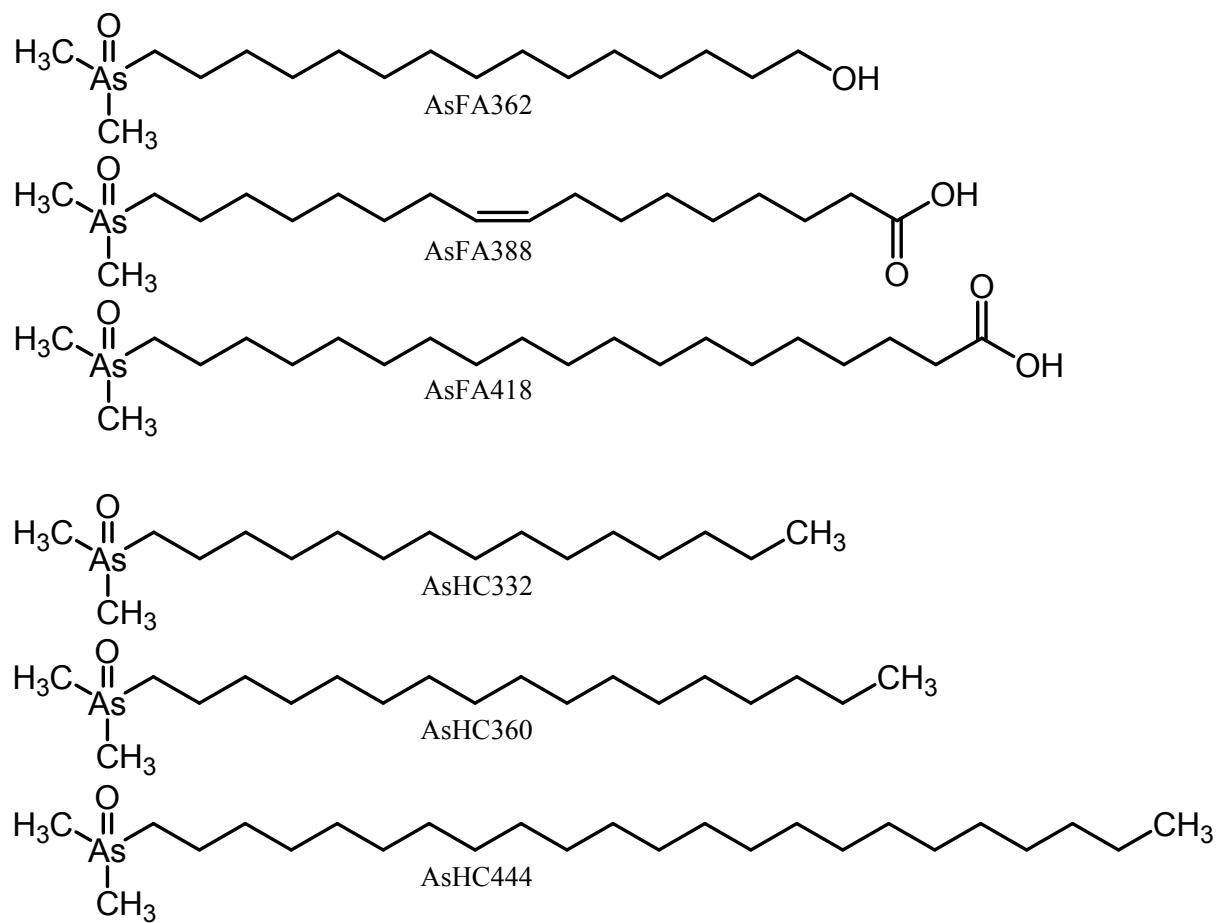


Fig. S1 Arsenic species synthesized in-house and referred to in this study (isomeric configuration and double bond position of AsFA388 were not determined).

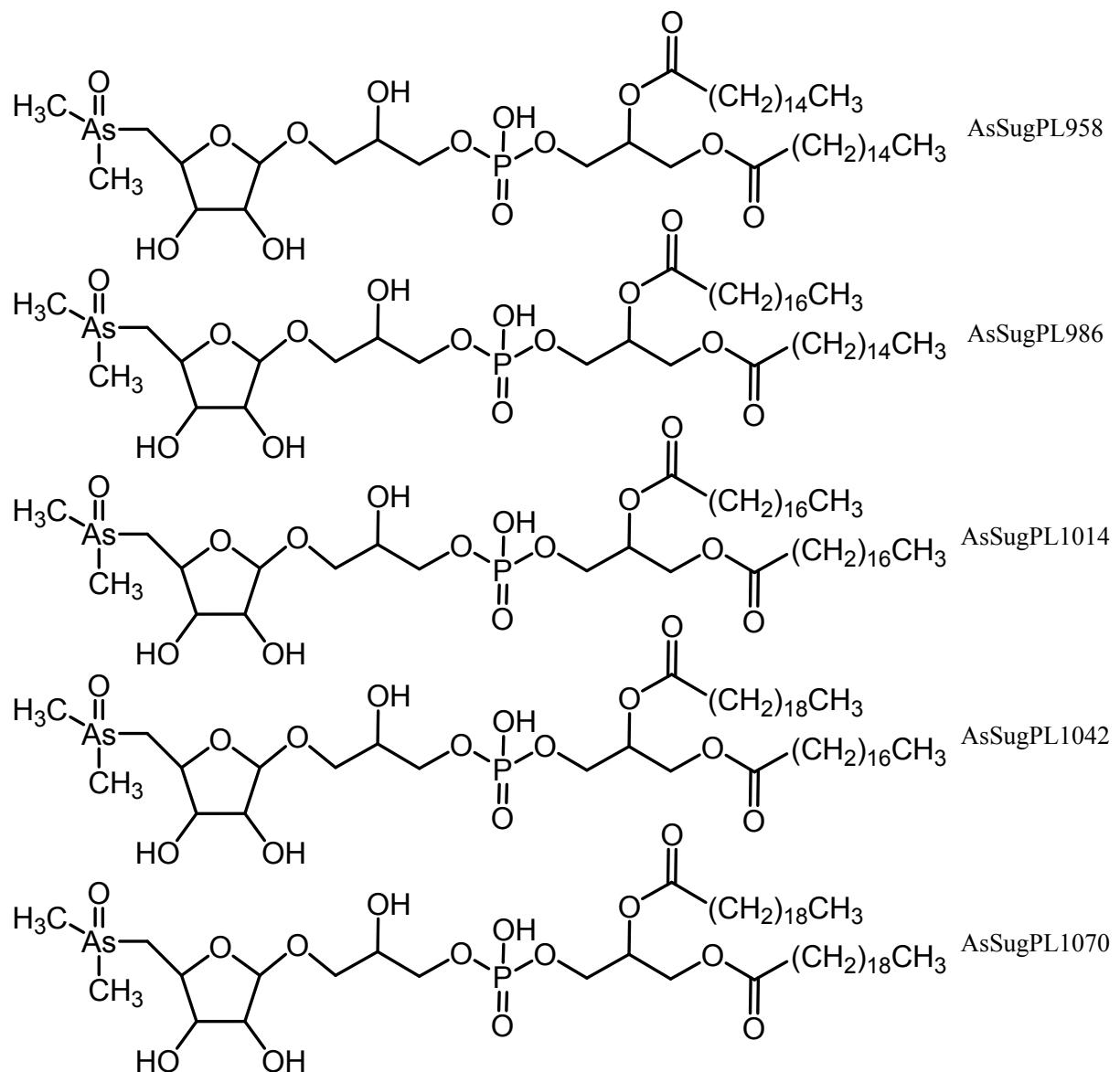


Fig. S2 Arsenic species previously described in NMIJ CRM 7405-a (Hijiki) and referred to in this study.