

Supplementary Materials

S1. Synthesis of Standard Compounds

S1.1. DMSP and D₆-DMSP

DMSP and D₆-DMSP as hydrochloride were synthesized according to Chambers [1] by bubbling gaseous hydrogen chloride through a solution of anhydrous acrylic acid (Fluka, Germany) and dimethyl sulfide (Sigma Aldrich, Germany) or D₆-dimethyl sulfide (Sigma Aldrich, Germany) in dichloromethane and subsequent recrystallization of the resulting white solid in MeOH/Et₂O.

S1.2. DMS-Ac and D₆-DMS-Ac

DMS-Ac and D₆-DMS-Ac as hydrobromide were synthesized according to Howard [2] by addition of commercially available dimethyl sulfide (Sigma Aldrich, Germany) and D₆-dimethyl sulfide (Sigma Aldrich, Germany), respectively, to a stirred solution of bromoacetic acid (Fluka, Germany) in dichloromethane. The resulting white solid was recrystallized in MeOH/Et₂O.

S1.3. Trimethylammonium Propionate

To a stirred solution of 100 mg of dimethylaminopropionic acid in 2 mL methanol were added 50 mg K₂CO₃ and 200 mg iodomethane (Sigma Aldrich, Germany) at room temperature. After two days, the resulting mixture was acidified by addition of aqueous hydroiodic acid (Alfa Aesar, Germany). Trimethylammonium propionate as hydroiodide was precipitated by addition of Et₂O as white solid, which was recrystallized in MeOH/Et₂O.

¹H-NMR (400 MHz, D₂O) δ ppm: 2.94 (2H, t, *J* = 7.68 Hz), 3.12 (9H, s), 3.64 (2H, t, *J* = 7.50 Hz); ¹³C-NMR (50 MHz, D₂O) δ ppm: 27.75, 52.75, 61.27, 172.98; ESI-MS *m/z* 132.12 [M + H]⁺; ESI-MS-MS (parent ion: *m/z* 132, collision energy: 15 eV) *m/z* 132.12 [M]⁺, 73.08 [M – C₃H₉N]⁺, 60.13 [C₃H₁₀N]⁺, 59.12, 58.11.

S1.4. Homarine (N-Methyl Picolinic Acid Hydroiodide)

N-methylpicolinic acid hydroiodide was synthesized by addition of 150 mg iodomethane (Sigma Aldrich, Germany) to a well stirred suspension of 100 mg picolinic acid (Alfa Aesar, Germany) in 2 mL propylene carbonate. After two days, 10 mL Et₂O were added, and the resulting yellow solid was recrystallized in MeOH/Et₂O (for synthesis see also [3]).

¹H-NMR (400 MHz, MeOD) δ ppm: 4.95 (3H, s), 8.06 (1H, t, *J* = 6.26 Hz), 8.44 (1H, d, *J* = 7.63 Hz), 8.51 (1H, t, *J* = 7.78 Hz), 8.85 (1H, d, *J* = 5.19 Hz); ¹³C-NMR (101 MHz, MeOD) δ ppm: 127.97, 130.51, 145.44, 146.71, 149.69, 163.90 (the signal of the methyl group was overlapped by the signals of the solvent and therefore not visible, cf. [4]); ESI-MS (positive) *m/z* 138.08[M + H]⁺; ESI-MS-MS (positive, parent ion: *m/z* 138, collision energy: 15 eV) *m/z* 138.08 [M + H]⁺, 124.09 [M – CH₃ + H]⁺, 106.08, 96.09, 94.11 [M – COOH + H]⁺, 78.08.

S1.5. Ethyl-3-hydroxy-5-methylthiopentanoate

In a three-necked flask (100 mL) with 5 g zinc powder 40 mL dry diethylether and 0.3 mL chlorotrimethylsilane were added under argon atmosphere. The resulting suspension was refluxed for 20 min. After dropwise addition of 3.3 g (19.8 mmol) ethyl-bromoacetate and 2.02 g (19.4 mmol) 3-(methylthio)propionaldehyde reaction mixture was refluxed over an additional 2.5 h. After cooling to room temperature 50 mL 3 M HCl were added and the resulting mixture was stirred for 20 min. The solution was then extracted with Et₂O and combined organic phases were washed with NaHCO₃ solution and water. Removal of the solvent gave a yellowish residue which was purified by silica gel column chromatography (petroleum ether/AcOEt = 4:1) to give 937 mg (25%) of the desired product as a colorless oil.

¹H-NMR (400 MHz, CDCl₃) δ ppm: 1.26 (3H, t, *J* = 7.14 Hz), 1.64–1.87 (2H, m), 2.10 (3H, s), 2.36–2.54 (2H, m), 2.55–2.71 (2H, m), 4.06–4.22 (3H, m); ¹³C-NMR (50 MHz, CDCl₃) δ ppm: 14.10, 15.46, 30.33, 35.53, 41.22, 60.69, 66.88, 172.62; EIMS *m/z* (relative intensity, 70 eV) 192.08 [M]⁺ (18), 174.07 [M – H₂O]⁺ (23), 144.08 (29), 129.03 [M – H₂O – C₂H₅O]⁺ (46), 107.07 (32), 100.04 (22), 99.04 (16), 98.03 (39), 87.02 (21), 85.02 (20), 75.03 [M – C₅H₉O₃]⁺ (20), 71.02 (28), 70.04 (22), 61.01 [M – C₆H₁₁O₃]⁺ (100).

S1.6. 3-Hydroxy-5-methylthiopentanoic Acid

To 475 mg Ethyl-3-hydroxy-5-methylthiopentanoate in a 4 mL screw cap vial, 2 mL 10% NaOH solution was added and shaken for 30 min at room temperature until the mixture became a homogeneous solution. After washing with Et₂O the yellowish solution was acidified with conc. HCl to pH 1. The aqueous phase was extracted five times with 2 mL Et₂O. Removal of the solvent gave 275 mg (63%) of an orange, viscous liquid.

¹H-NMR (400 MHz, CDCl₃) δ ppm: 1.68–1.91 (2H, m), 2.11 (3H, s), 2.47–2.58 (2H, m), 2.58–2.67 (2H, m), 4.15–4.25 (1H, m), 5.90 (1H, br. s); ¹³C-NMR (50 MHz, CDCl₃) δ ppm: 15.44, 30.32, 35.29, 41.07, 66.99, 176.91; ESI-MS (negative) *m/z* 163.09 [M – H][–].

S1.7. D₃-Gonyol (as Hydroiodide)

100 mg of 3-hydroxy-5-methylthiopentanoic acid were dissolved in 1 mL acetone. After addition of 0.05 mL Iodomethane-d₃ the reaction mixture was stirred for 12 h at room temperature while an orange oily liquid precipitated out of the solution. After removal of the solvent under reduced pressure and reprecipitation of the resulting residue in MeOH/Et₂O 145 mg (77%) of D₃-gonyol hydroiodide were obtained.

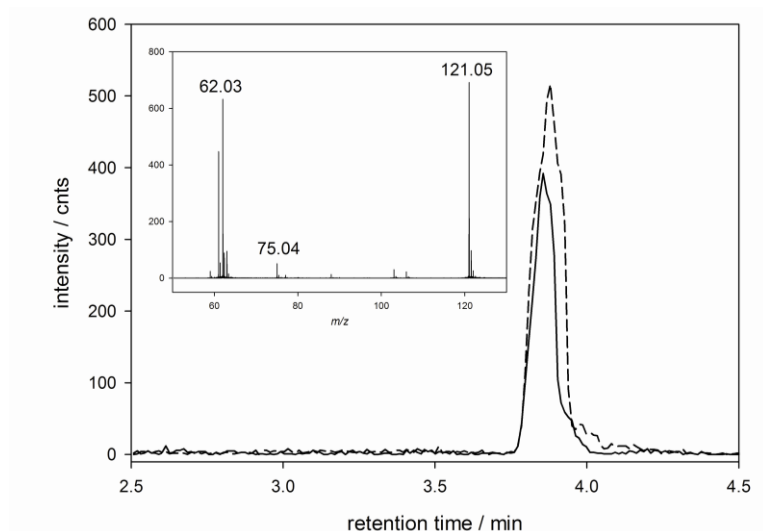
¹H-NMR (400 MHz, MeOD) δ ppm: 1.89–2.01 (1H, m), 2.07–2.17 (1H, m), 2.47–2.60 (2H, m) 2.94, 2.95 (3H, ss), 3.37–3.53 (2H, m), 4.10–4.19 (1H, m); ¹³C-NMR (101 MHz, MeOD) δ ppm: 25.84, 26.19, 31.98, 42.40, 42.77, 67.83, 174.78; ESI-MS (positive) *m/z* 182.02 [M + H]⁺; ESI-MS-MS (positive, parent ion: *m/z* 182, collision energy: 15 eV) *m/z* 182.02 [M + H]⁺, 117.11 [M + H – C₂H₃D₃S]⁺, 99.10 [M + H – H₂O – C₂H₃D₃S]⁺, 89.07, 87.10, 75.09, 71.10, 66.09 [C₂H₄D₃S]⁺, 57.08, 55.10.

S2. Identification of Different Osmolytes

Chemical structures of all previously unknown osmolytes in cell extracts were verified by ESI-MS-MS experiments and co-injection with standard compounds. Collision energy for all ESI-MS-MS experiments was set to 15 eV. In the following ion traces of investigated osmolytes of cell extracts (solid lines), standard compounds (dashed lines) and corresponding mass spectra are shown.

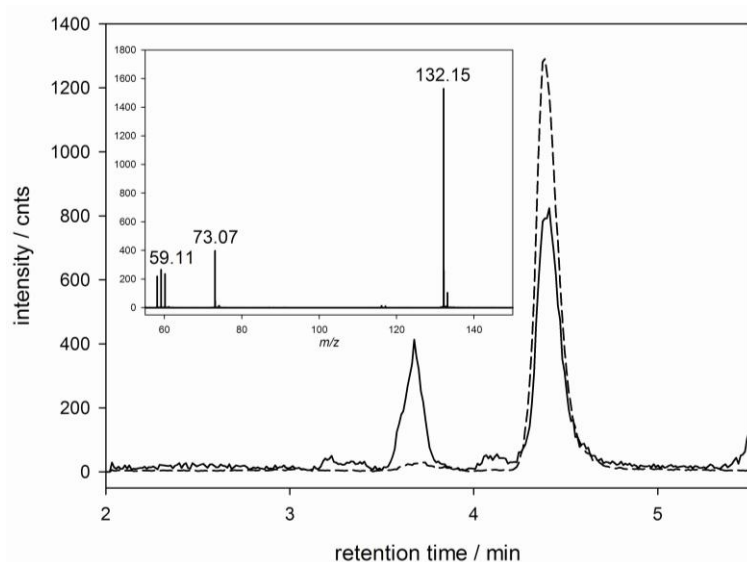
S2.1. DMS-Acetate

Figure S1. Ion traces ($m/z = 121$) of dimethylsulphonioacetate (DMS-Ac) in cell extracts (—), corresponding standard compound (--) and ESI-MS-MS spectrum of DMS-Ac (parent ion: $m/z = 121$).



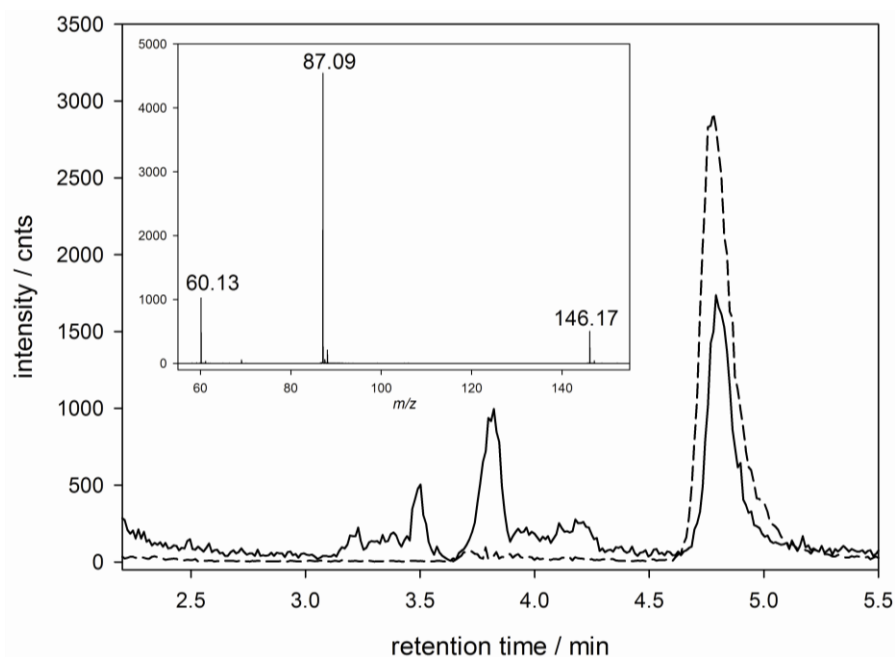
S2.2. TMAP

Figure S2. Ion traces ($m/z = 132$) of trimethylammoniumpropionate (TMAP) in cell extracts (—), corresponding standard compound (--) and ESI-MS-MS spectrum of TMAP (parent ion: $m/z = 132$).



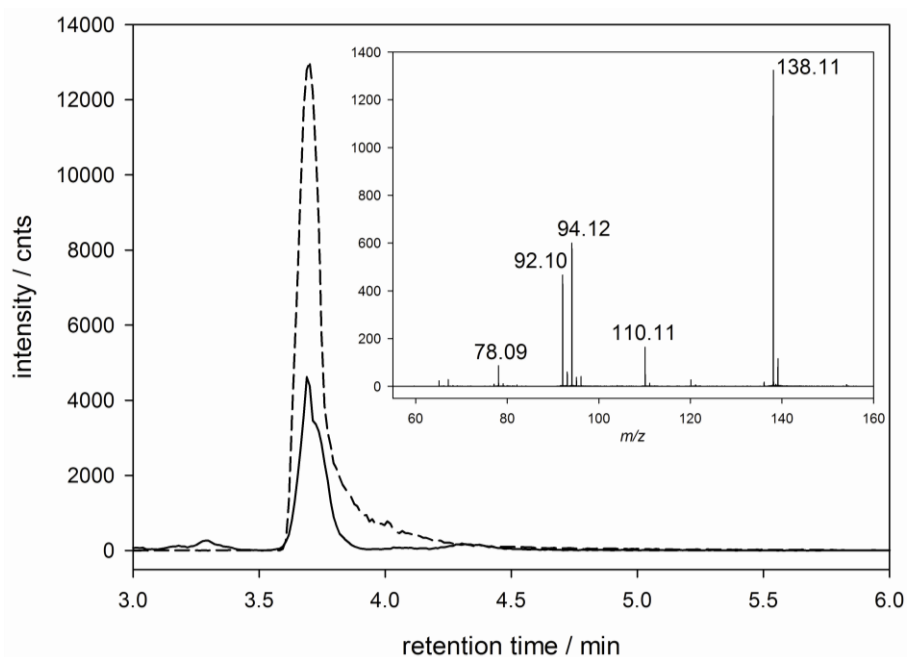
S2.3. TMAB

Figure S3. Ion traces ($m/z = 146$) of trimethylammoinimbutyrate (TMAB) in cell extracts (—), corresponding standard compound (--) and ESI-MS-MS spectrum of TMAB (parent ion: $m/z = 146$).



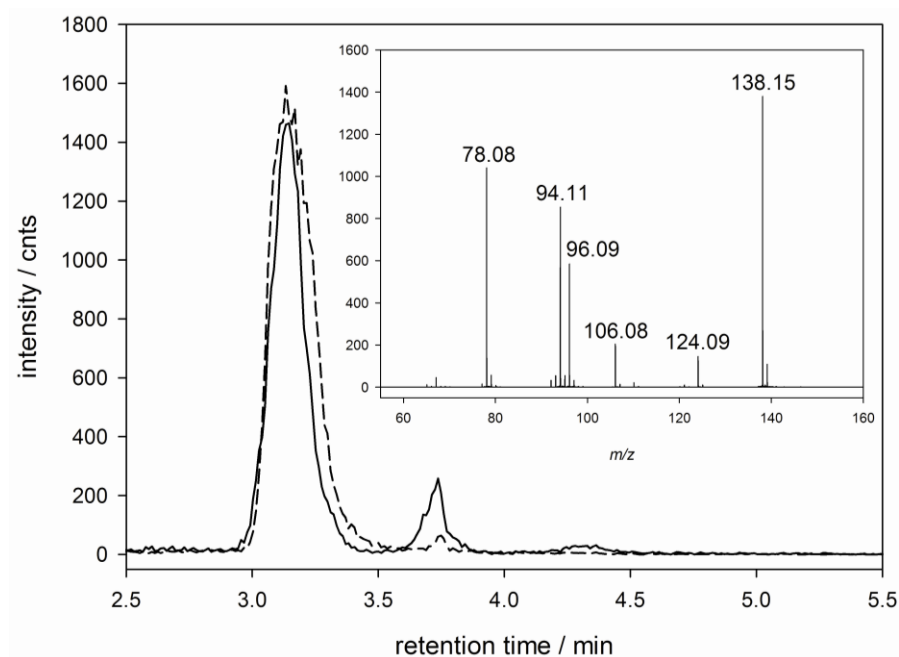
S2.4. Trigonelline

Figure S4. Ion traces ($m/z = 138$) of trigonelline in cell extracts (—), corresponding standard compound (--) and ESI-MS-MS spectrum of trigonelline (parent ion: $m/z = 138$).



S2.5. Homarine

Figure S5. Ion traces ($m/z = 138$) of homarine in cell extracts (—), corresponding standard compound (---) and ESI-MS-MS spectrum of homarine (parent ion: $m/z = 138$).

S3. Growth Curves (*in Vivo* Chlorophyll-A Fluorescence Data)

Figures S6 and S7 show measured *in vivo* chlorophyll-A fluorescence of *E. huxleyi* and *P. minimum* over the growth curve. Last data point represents the day of sample collection and extraction of the algae.

Figure S6. *In vivo* chlorophyll-A fluorescence during growth curve of *E. huxleyi* RCC1216 cultures grown in HW sea salt medium with salinities of 16‰ (—), 20‰ (—), 26‰ (—), 30‰ (—), 34‰ (—) and 38‰ (—); error bars represent standard deviation between biological replicates, last data point is day of sample collection and cell extraction.

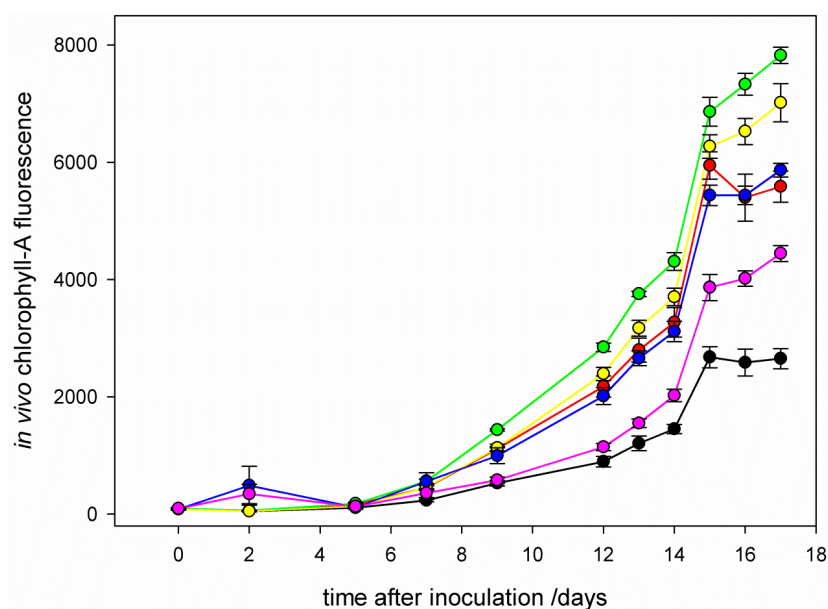
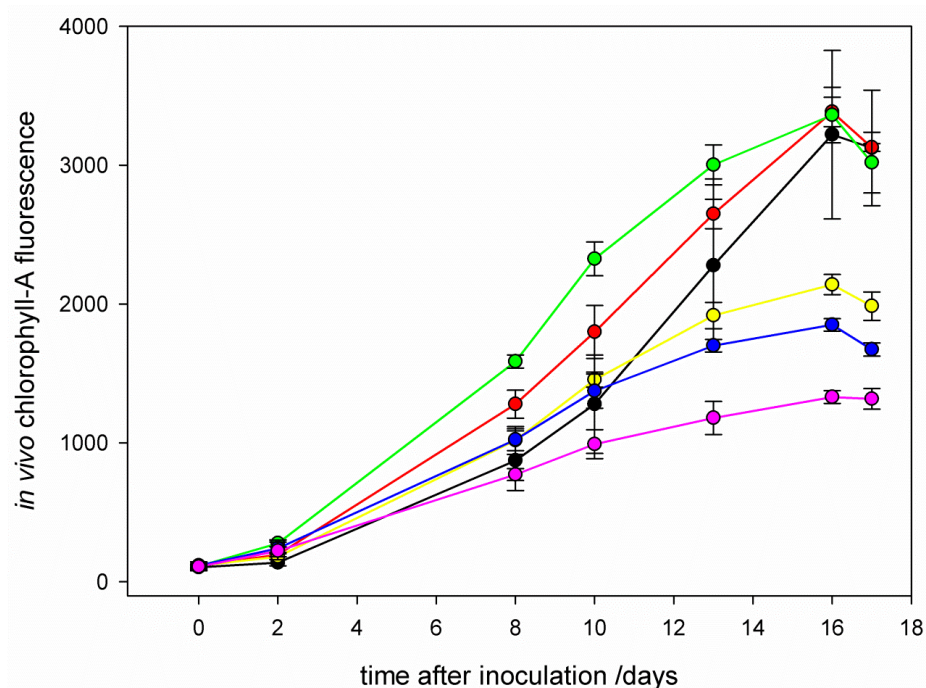


Figure S7. *In vivo* chlorophyll-A fluorescence during growth curve of *P. minimum* cultures grown in artificial seawater with salinities of 16‰ (—), 20‰ (—), 26‰ (—), 28‰ (—), 32‰ (—) and 36‰ (—); error bars represent standard deviation between biological replicates, last data point is day of sample collection and cell extraction.



References

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