

ADDITIONAL FILE 1

Delineating the molecular responses of a halotolerant microalga using integrated omics approach to identify genetic engineering targets for enhanced TAG production

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Fig. S1: Heat maps showing z-scores of discriminatory metabolite entities in ASW cells compared to control culture. X-axis represents the replicates of the culture (A); (Control – lane (1-6) - red bar (B); ASW – lane (7-12) The color scheme through signifies the elevation and reduction in metabolite concentration in ASW compared to control : dark blue: lowest; dark red: highest.

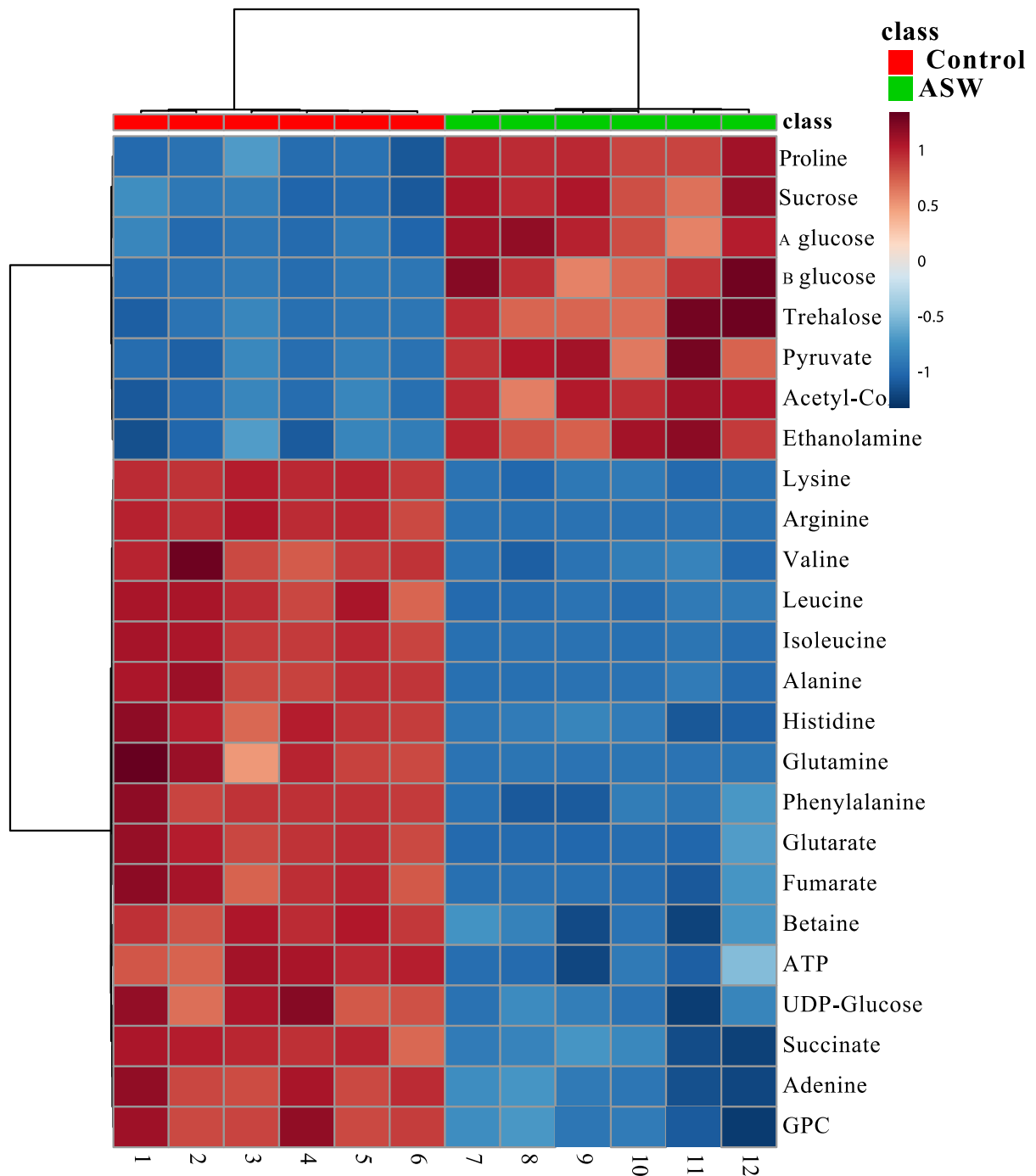


Fig. S2: Similarity search for *Scenedesmus* sp. IITRIND2 against freshwater and marine microalgal species based on the identified proteins using proteomics studies. The functional annotation and grouping of the identified proteins was done by QuickGo, EMBL-EBI (<https://www.ebi.ac.uk/QuickGO/>) using Explore biology tool. The annotation and proteomics analysis suggested that the identified proteins of *Scenedesmus* sp. IITRIND2 belonged to 15 different algae which comprises of both fresh water and marine species. For fresh water species, the maximum hits were related to *Desmodesmus communis* (12 %), followed by *Chlamydomonas reinhardtii* (11 %) and *Ettlia pseyudoalveolaris* (10 %) respectively. On the other hand, for marine algae, maximum similarity was recorded with *Ostreococcus tauri* (8.33 %) respectively.

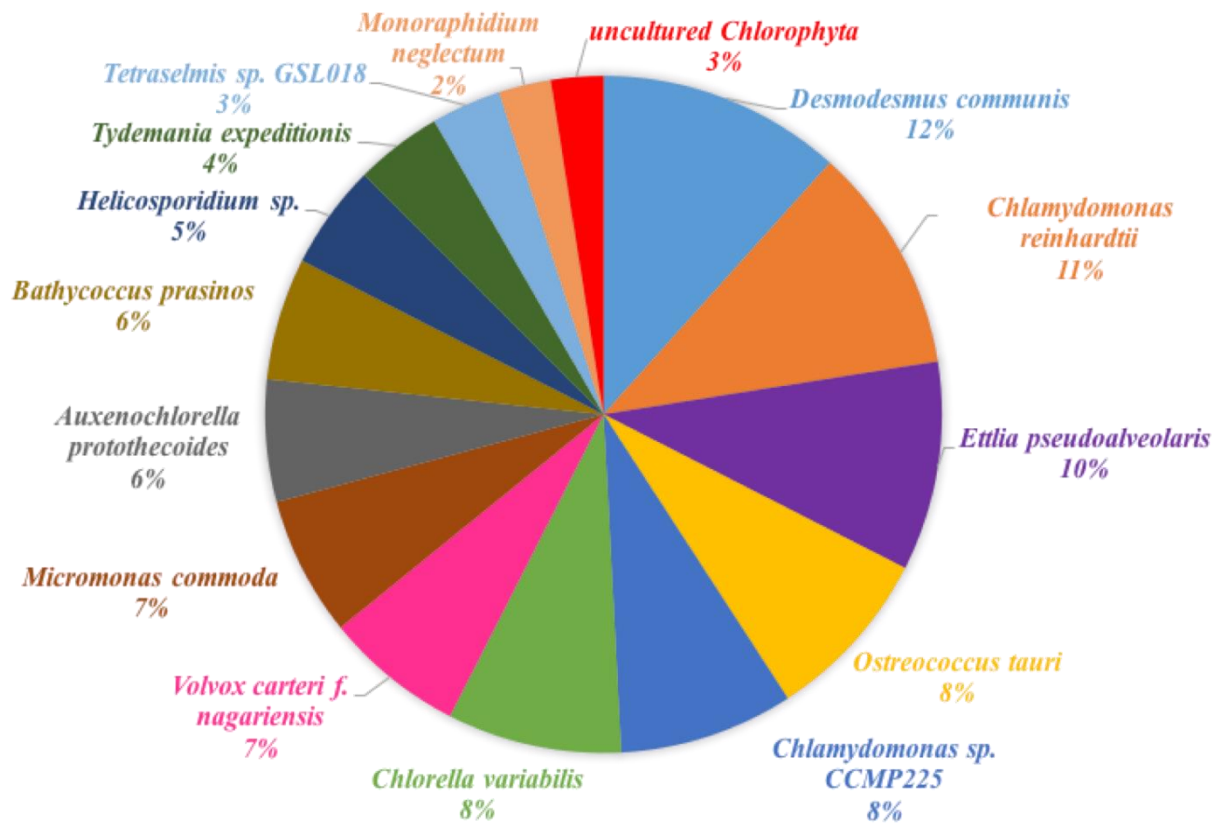


Table S1: List of primers used in the expression analysis (RT-PCR)

Gene	Forward	reverse	Tm	GC	Reference
Beta Actin	ACATCAAGGAGAAGCTGGCCT A	ATGTCGACGTCGCACTTCATGA	F 54.8	50	-
			R 54.8	50	
DGAT-3280	GGCACAAAGAGTTCACCGT	ACAAACTTGAGGTGGGTG	F 51.1	53	[1]
			R 48	50	
ME-3137	CCCTCTCGTTCCCCTTTTATT	AAATGCTGACGCAAGTGTGA	F 52.4	48	[1]
			R 49.7	45	
P5CS	GTGCCCATCGGCGTGCTTCT	CGTGTTGCGCTTGATGTGGC	F 57.9	65	[2]
			R 55.9	60	
BC	TGCGATTGGGTATGTGGGGGT	ACCAGGACCAGGGCGGAAAT	F 56.3	57	[3]
			R 55.9	60	
SAD	TCCAGGAACGTGCCACCAAG	GCGCCCTGTCTTGCCCTCAT	F 55.9	60	[3]
			R 57.9	65	
PGAT	GGATAAGAGCGGCACAAGGA	GAAGGGCGAGATTGGAATGA	F 53.8	55	[4]
			R 51.8	50	
LIP	GGCCTCAAAGCCACCAGTAC	GGCAGTGCACATGTTGCAG	F 55.9	60	[4]
			R 53.2	58	
SS	CAGGCAAGGATACATCTACTG	TACTGCCCAACCATCTCATC	F 55.5	47.6	-
			R 56.9	50	
AGP-L	CCATGAGCAACTGCATCAAC	GGTTGAGCGAGGTGGAGTT	F 51.8	50	[5]
			R 53.2	58	
Psac	GAACATCACCACCACCAGGA	CGGTGCTTGGCTTTTAGTTTG	F 53.8	55	[6]
			R 52.4	48	
CA	TGAAGGAGGGCTCTGATGAT	GTTTGCGAATGAGATGGTGT	F 51.8	50	[7]

Table S2: List of lipids along with their respective chemical shifts and fold change as evaluated using Bruker Top spin 3.5 (* indicates the chemical shift taken for fold change estimation).

S.No.	Lipids	Chemical shift (ppm)	Fold change
1	PUFA	2.76 (d)	8.7
2	Phosphatidylcholine	3.38 (s)	3.05
3	Phosphatidylethanolamine	3.22 (s)	4.09
4	Total phospholipids	3.95 (s)	8.13
5	Triglycerides	4.12 (q)*, 4.27 (q), 5.25 (s)	17.2
6	Fatty acid residues	5.32 (m)	10.05
7	MGDG	3.88 (s)	1.9
8	Omega3 PUFA	0.85 (t)*, 0.95 (t)	6.03

Table S3: List of metabolites along with their respective chemical shifts and metabolic fold change patterns as a consequence salt stress. One-way ANOVA followed by post-hoc Tukey's HSD was conducted to determine significant ($p < 0.001$) metabolic changes.

Metabolite Name	Assignment	Chemical shifts (δ) in ppm	Fold change in treated cells
Amino acids			
Leucine	δ -CH ₃ δ -CH ₃	0.95 (d) 0.96 (d) ^ε	-1.2
Isoleucine	γ -CH ₃ δ -CH ₃	0.93 (t) ^ε 1.00 (d)	-1.3
Valine	γ -CH ₃ γ -CH ₃	0.98 (d) 1.03 (d) ^ε	-1.3
Arginine	γ -CH ₂	1.68 (m) ^ε	-2.5
Lysine	δ -CH ₂	1.69 (m) ^ε	-1.3
Alanine	β -CH ₃ α -CH	1.47 (d) ^ε 3.79 (q)	-1.5
Proline	γ -CH ₂ γ -CH ₂ $\frac{1}{2}$ β -CH ₂ $\frac{1}{2}$ β -CH ₂ $\frac{1}{2}$ β -CH ₂	1.99 (m) 2.00 (m) 2.06 (m) 3.32 (m) ^ε 3.41 (dt)	+10.2
Histidine	C4H-ring	7.71 (d)	-1.2
Glutamate	β -CH ₂ γ -CH ₂	2.11 (m) ^ε 2.34 (m)	-1.1
Glutamine	β -CH ₂ γ -CH ₂	2.12 (q) 2.44 (m) ^ε	-1.1
Sarcosine [#]	N-CH ₃	2.71 (s)	+1.4
Glycine	α -CH ₂	3.56 (s)	-
Tyrosine	C2H & C6H C3H & C5H	6.88 (d) ^ε 7.18 (d)	-
Phenylalanine	C2H & C6H C4H C3H & C5H	7.31 (m) 7.37 (m) 7.43 (m) ^ε	-1.1
γ -glutamyl-phenylalanine	C2H, C6H & C4H C3H & C5H	7.3 (m) ^ε 7.4 (m)	-
Organic acids			
Lactate	β -CH ₃ α -CH	1.32 (d) ^ε 4.10 (m)	-1.4
Acetate	CH ₃	1.91 (s)	-1.5
Aspartate	C6H, C6H	2.8 (m) ^ε , 2.66 (q)	-1.6
Pyruvate	γ -CH ₃	2.4 (s)	+1.5
Glutarate	β , δ -CH ₂	2.16 (t)	-1.2
Succinate	α , β -CH ₂	2.39 (s)	-1.9
Citrate	$\frac{1}{2}$ γ -CH ₂ $\frac{1}{2}$ γ -CH ₂	2.52 (d) ^ε 2.69 (d)	-
Fumarate	CH	6.51 (s)	-1.6
Formate	CH	8.44 (s)	-
Carbohydrates/sugar			
Sucrose	C10H C12H C13H	3.46 (t) ^ε 3.55 (dd) 3.66 (s)	+6.1

	C11H C17H & C19H C5H & C9H C4H C3H C7H	3.75 (m) 3.77 (m) 3.81 (dd) 4.04 (t) 4.21(d) 5.40(d)	
α -Glucose	C4H	3.39 (m)	+3.8
β -Glucose	C5H C3H C1H	3.45 (m) 3.47 (m) [€] 5.22 (d)	+2.6
Mannose/Trehalose	C1H	5.19 (d)	+1.9
Glucose-1-phosphate	C1H	5.55 (d,d)	-
Phosphagen			
Choline/PC	N-(CH ₃) ₃	3.20 (s)	-2.3
GPC	N-(CH ₃) ₃	3.22 (s)	-1.6
Osmolytes			
Glycerol	$\frac{1}{2}$ γ -CH ₂ $\frac{1}{2}$ γ -CH ₂	3.63 (d) [€] 3.65 (d)	-
Betaine	N-(CH ₃) ₃ β -CH ₂	3.26(s) [€] 3.91 (s)	-
TMAO	N-(CH ₃) ₃	3.27(s) [€]	+3.2
Nucleotides			
Adenine	C2H C6H	8.19 (s)	-2.6
ATP	C7H C12H C2H	8.61 (s) [€] 8.25 (s) 6.13 (d)	-1.2
NAD ⁺	C28H	6.08 (m)	-1.3
Others			
Ethanol	CH ₃	1.17 (t)	-
Acetyl choline	S-(CH ₂) ₂	3.2 (s)	+1.6
3-hydroxy- isovalerate	γ -CH ₃	1.24 (s)	-
Acetone	CH ₃	2.22 (s)	-
UDP-Glucose	C22H	6 (m)	-2.1
Ethanolamine	N-CH ₂	3.13(m)	+2.2
Methanol	CH ₃	3.34 (s)	-

Note: All the values of the metabolites were statistically significant with p value 0.001 respectively. “€” represents the metabolite peak used for evaluating the quantitative difference as represented here fold changes in the case of multiple signals/chemical shift values.

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