1   Research Article

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3   A Data-Independent-Acquisition-based proteomic approach towards

4   understanding the acclimation strategy of *Microchloropsis gaditana*

5   CCMP526 in hypersaline conditions

6

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32 **ABSTRACT**

33

34 Salinity is one of the significant factors that affect growth and cellular metabolism, including

35 photosynthesis and lipid accumulation, in microalgae and higher plants. *Microchloropsis*

36 *gaditana* CCMP526 can acclimatize to different salinity levels by accumulating compatible

37 solutes, carbohydrates, and lipid as an energy storage molecule. We used proteomics to

38 understand the molecular basis for acclimation of *M. gaditana* to increased salinity levels (55

39 and 100 PSU (Practical Salinity Unit). Correspondence analysis (CA) was used for

40 identification of salinity-responsive proteins (SRPs). The highest number of altered proteins

41 was observed in 100 PSU. Gene Ontology (GO) enrichment analysis revealed a separate path

42 of acclimation for cells exposed to 55 and 100 PSU. Osmolyte and lipid biosynthesis was up-

43 regulated in high saline conditions. However, concomitantly lipid oxidation pathways were

44 also up-regulated at high saline conditions, providing acetyl-CoA for energy metabolism

45 through the TCA cycle. Carbon fixation and photosynthesis were tightly regulated, while

46 chlorophyll biosynthesis was affected under high salinity conditions. Importantly, temporal

47 proteome analysis of salinity-challenged *M. gaditana* revealed vital salinity-responsive

48 proteins which could be used for strain engineering for improved salinity resistance.

49

50 **Introduction**

51

52 Biofuel sourced from algae is considered as an effective renewable alternative to fossil fuels

53 (Georgianna & Mayfield, 2012). Microalgae can grow in a variety of environmental

54 conditions all year-round and produce valuable products such as biofuel, animal feeds, and

55 pharmaceuticals (Brennan & Owende, 2010; Doan et al., 2011). Moreover, many species can

56 accumulate high amounts of lipid under stress, and this phenomenon has been extensively

57 researched in promising biofuel candidates under various stress conditions (Corteggiani

58 Carpinelli et al., 2014; Huertas et al., 2000; Simionato et al., 2011). However, limitations

59 such as low lipid productivity and high costs associated with downstream processing have

60 hindered the commercialization of algae-based biofuel (Wijffels & Barbosa, 2010). A simple

61 approach for enhancing productivity coupled with high growth rate would be to manipulate

62 the environmental parameters. Salinity is one of the primary growth influencing factors for

63 marine microalgae, which could be controlled in an open race-way pond (Kim et al., 2016;

64 Perrineau et al., 2014).

65

66 Extensive research has been done on increasing lipid accumulation in various microalgal

67 species using salinity stress (Kim et al., 2016; Perrineau et al., 2014). Notably, the

68 halotolerant alga *Dunaliella* sp. is known to perform well in high saline conditions. It

69 accumulates a high amount (~5 M) of glycerol when exposed to hypersaline conditions

70 (Takagi & Yoshida, 2006). Lipid accumulation was enhanced to 59.4% in *Chlamydomonas*

71 sp. JSC4 by gradually increasing the salinity levels up to 2% (w/v) of sea salt (Ho et al.,

72 2017). Accumulation of lipid or glycerol in such conditions involves a major metabolic shift

73 in re-directing the carbon flux, which causes alteration in the abundance of proteins/enzymes

74 involved in the metabolism of lipid or glycerol.

75

76 Appropriately, various proteomic studies can be performed to understand the acclimatization

77 process of microalgae in high saline conditions. The proteome of the halotolerant alga

78 *Dunaliella* sp. has been studied to understand the molecular basis behind tolerance to high

79 saline conditions (Katz et al., 2007; Wei et al., 2017). Sithtisarn et al. (2017) performed

80 comparative proteome analysis of a control, and a salinity tolerant strain of *Chlamydomonas*

81 *reinhardtii* to understand the salinity tolerance mechanism involved. Alterations in proteome

82 of terrestrial plants have also been extensively studied (Aghaei et al., 2008; Silveira &

83 Carvalho, 2016). The response to salinity appears similar in both microalgae and higher

84 plants. Several biosynthetic pathways were affected under high salinity conditions, including

85 photosynthesis, lipid, carbohydrate, and amino acid metabolism (Pandit et al., 2017).

86

87 *Microchloropsis gaditana* CCMP526, previously known as *Nannochloropsis gaditana*

88 CCMP526, is one of the six algal species from the genus *Nannochloropsis* (Fawley et al.,

89 2015). It is a marine microalga known for high oil accumulating capability and especially its

90 eicosapentaenoic acid (EPA) content. Physiological alterations in the lipid and carbohydrate

91 accumulation have been observed in high salinity conditions, which prompted us to

92 investigate the genes and proteins involved in the acclimatization mechanism. A systematic

93 investigation using NGS-based transcriptomic (RNA-Seq) approach was performed to

94 identify the molecular players in salinity tolerance. The results showed active shunting of

95 carbon flux towards lipid accumulation and osmolyte biosynthesis. In this work, we aim to

96 identify the salinity-responsive proteins (SRPs) using a DIA (data-independent acquisition)-

97 proteomic approach. The traditional proteomic analysis uses data-dependent acquisition

98 (DDA), which is often limited by the number of proteins and peptides that can be analyzed in

99 one run. In DIA analysis, all peptides within a defined *m/z* range are fragmented, resulting in

100   an increase in the number quantifiable peptides and a more accurate peptide quantification

101   (Doerr, 2014).

102

103   Temporal proteomic analysis was performed on *M. gaditana* subjected to different salinity

104   levels (38, 55 and 100 PSU). Time points and salinity levels for sampling were chosen based

105   on the lipid and carbohydrate accumulation from our previous physiological analysis.

106   Systematic analysis of the temporal proteome data revealed vital salinity-responsive proteins

107   (SRPs) and the dynamics of essential proteins involved in the cellular metabolic shift towards

108   acclimatization.

109

110   **Materials and methods**

111

112   ***Cultivation and harvesting***

113

114   *M. gaditana* was cultivated in 0.2 µm-filtered sea water (collected from the Gippsland Lakes,

115   Gippsland, Victoria, Australia), supplemented with Guillard's f/2 nutrients [1] and 17 mM

116   sodium nitrate, at 25°C using 500 ml glass bottles (Schott Duran, Germany). Cultures (300

117   ml) were mixed by bubbling with sterile air (0.2 µm filtered) supplied at a flow rate of 2.5 L

118   min^{-1}. Illumination was provided at 150 µmol · photons · m^{-2} · s^{-1} (Philips, TLD36W,

119   Amsterdam, The Netherlands) with a light/dark cycle of 12/12 h. Sodium chloride was added

120   to the existing f/2 media (control, 38 PSU) to make f/2 media of different salinities (55 and

121   100 PSU). A portable refractometer (RHS-10ATC) was used to assess the salinity of the

122   culture media. Growth curve experiment (cell count and OD_{685}) was performed in standard

123   conditions (38 PSU, 25°C, and 150 µmol · photons · m^{-2} · s^{-1}) to determine the growth phases

124   in *M. gaditana* (data not shown). Cells at mid exponential phase were inoculated into f/2

125   media of different salinities at a concentration of approximately $4x10^6$ cells/ml. Cells were

126   then sampled from three independent cultures at specific time points (0, 1, 6, 24 and 72 h)

127   that were chosen from our earlier physiological study [2]. The samples were centrifuged (4000

128   xg, 4°C, 5 min) in an Heraeus Multifuge model 3SR Plus (thermos Scientific, Australia) and

129   the resulting cell pellet was washed thrice with sterile distilled water to remove any residual

130   salt from the growth medium. The pellet was then stored at -80°C until further processing.

131

132   *Protein isolation and Sample preparation for mass spectrometry*

133

134   Protein was isolated using a Bioline II DNA/RNA/Protein extraction kit (Bioline, Australia)

135   following the manufacturer's protocol. The isolated protein was quantified using the

136   absorbance at 280 nm (Thermo Scientific, Australia). The samples were stored in -80°C until

137   further processing. Approximately 50 mg of protein was denatured using TCEP (Thermo

138   Scientific, Cat. #77720) to a final concentration of 10 mM. The solution was then incubated

139   at 50-65°C for 30 min. Chloroacetamide (CAA) was added to a final concentration of 40 mM

140   to alkylate the reduced protein. The mixture was incubated at room temperature in the dark

141   for 20 min. Trypsin at a dilution of 1:100 was added and incubated overnight at 37°C with

142   shaking. The reaction was stopped by adding 1% formic acid (FA) (Wei et al.). The samples

143   were then desalted using Ziptips (Agilent, OMIX-Mini Bed 96 C18, A57003MBK). The

144   residual acetonitrile (ACN) was removed using a vacuum concentrator. The sample was

145   dissolved into 20 ml of 0.1% FA and sonicated in a water bath for 10 min. Any insoluble

146   precipitates were removed by centrifuging for 5 min. iRT (indexed retention time) peptides

147   were added before transferring the sample into an MS vial.

148

149   *Mass spectrometry Data Acquisition*

150

151   *Data-dependent acquisition (DDA) mass spectrometry*

152

153   Using a Dionex UltiMate 3000 RSLCnano system equipped with a Dionex UltiMate 3000 RS

154   autosampler, the samples were loaded via an Acclaim PepMap 100 trap column (100 µm x 2

155   cm, nanoViper, C18, 5 µm, 100å; Thermo Scientific) onto an Acclaim PepMap RSLC

156   analytical column (75 µm x 50 cm, nanoViper, C18, 2 µm, 100å; Thermo Scientific). The

157   peptides were separated by elution with increasing concentrations of 80% ACN / 0.1% FA at

158   a flow rate of 250 nl/min for 158 min and analyzed with an Orbitrap Fusion Tribrid mass

159   spectrometer (Thermo Scientific). Each cycle was set to a fixed cycle time of 4 sec consisting

160   of an Orbitrap full ms1 scan (resolution: 120.000; AGC target: 1e6; maximum IT: 54 ms;

161   scan range: 375-1575 m/z) followed by several Orbitrap ms2 scans (resolution: 30.000; AGC

162   target: 4e5; maximum IT: 118 ms; isolation window: 1.4 m/z; HCD Collision Energy: 32%).

163   To minimize repeated sequencing of the peptides, dynamic exclusion was set to 15 sec, and

164   the 'exclude isotopes' option was activated. The raw and analyzed DDA data files have been

165   deposited to the ProteomeXchange Consortium

166   (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository (Vizcaíno et

167   al., 2016)[3] with the dataset identifier PXD017164 (Username: reviewer64456@ebi.ac.uk,

168   Password: 1MhuJS1T).

169

170   ***Quantification of proteins using data-independent acquisition (DIA) mass spectrometry***

171

172   The identical instrument setup as described above (Dionex UltiMate 3000 LC system coupled

173   to an Orbitrap Fusion Tribrid mass spectrometer) was used to quantify proteins using data-

174   independent acquisition (DIA). 25 sequential DIA windows with an isolation width of 24 m/z

175   between 375 - 975 m/z were acquired (resolution: 30.000; AGC target: 1e6; maximum IT: 54

176   ms; HCD Collision energy: 32%; scan range: 200-2000 m/z) following a full ms1 scan

177   (resolution: 120.000; AGC target: 1e6; maximum IT: 54 ms; scan range: 375-1575 m/z). A

178   158 min gradient of increasing concentrations of 80% ACN / 0.1% FA was used to separate

179   the peptides for the DIA acquisition. The raw and analyzed DIA data files have been

180   deposited to the ProteomeXchange Consortium

181   (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository (Vizcaíno et

182   al., 2016) with the dataset identifier PXD017297

183   (Username: reviewer44761@ebi.ac.uk, Password: Yszk3q3x).

184

185   ***Mass spectrometric data analysis***

186

187   Acquired DDA .raw files were searched against the *N. gaditana* UniProtKB/SwissProt

188   database (v2017_07) using Byonic (Protein Metrics) embedded into Proteome Discoverer

189   (Thermo Scientific) to obtain peptide sequence information. Only peptides identified at a

190   false discovery rate (FDR) of 1% based on a decoy database were considered for further

191   analysis. Spectronaut Orion (Biognosys) was used to create the corresponding spectral library

192   as well as to evaluate all DIA data using in-house parameters. To correct for differences in

193   sample density and loading, the peak areas for each peptide were normalized based on the

194   assumption that on average, a similar number of peptides are up- and down-regulated. Two-

195   sided t-tests were used to calculate p-values and the FDR for each time point (0, 1, 6, 24 and

196   72 h) and salinity level (55 and 100 PSU) against control (38 PSU), based on multiple

197   hypotheses testing corrections by Benjamini-Hochberg method (implemented in R). GO

198   terms were enriched using a Fisher exact test with p-value <0.05.

199

200   **Results and discussions**

201

202   *General description of the proteomics results*

203

204   DIA based proteomic analysis was employed to investigate the temporal acclimatization

205   strategy of *M. gadit*ana at different salinity levels (55 and 100 PSU) compared to controls at

206   38 PSU, and at various time points (0, 1, 6, 24 and 72 h). These time points represent the

207   critical stages of change in cellular state in regard to alterations in photosynthetic rate,

208   carbohydrate, and lipid accumulation (Karthikaichamy et al., 2018). Changes in protein level

209   are represented as average $\log_2$(fold change) compared with the control (38 PSU) sample at

210   corresponding time points (figure 1A). On average, the proteins were up-regulated in cells

211   exposed to 100 PSU (except at 0 and 24 h). A similar trend was observed in cells in 55 PSU,

212   except at 6h, where the average $\log_2$(fold change) value indicated significant down-regulation

213   of proteins (p-value=0.000002). Noticeably, the average $\log_2$(fold change) in 100 PSU was

214   significantly higher than 55 PSU at 72 h (p-value <0.000001). This sudden up-regulation of

215   protein during the later phase of growth in 100 PSU indicates a strong response towards

216   acclimation. To identify the proteins that were expressed in all timepoints, Venn diagram was

217   constructed (figure 1B). A total of 1874 proteins were expressed across all the time points,

218   and these proteins were selected for temporal analysis.

219

220   **Figure 1. A.** Differential protein expression at different time points and salinity levels (55

221   and 100 PSU) compared to control (38 PSU). Values plotted are the average expression at a

222   particular time point, and error bars indicate SEM; **B.** Venn diagram showing the overlapping

223   differentially abundant proteins in different time points.

224

225   The proteins that satisfied the cut-off criteria (p-value<0.05 and absolute $\log_2$(fold change)

226   >1) in at-least single time-point were selected for further analysis. *M. gaditana* grown in 55

227   PSU had 456 significantly expressed proteins compared to 1359 proteins in cells exposed to

228   100 PSU relative to control condition (38 PSU). The highest number of proteins that were

229   differentially expressed were in cells grown under 100 PSU at 72 h (264 down-regulated and

230   1110 up-regulated proteins), suggesting a major proteomic shift towards acclimation in high

231   saline conditions. There was also a robust initial response to change in salinity. Ideally the

232   protein expression levels should remain the same at 0h, irrespective of the salinity. However,

233   we observed differential expression of proteins at 0h in 55 and 100 PSU. This is explained by

234   the time required for harvesting and processing the cells for proteomics experiment. In 55 and

235   100 PSU, 157 and 454 proteins were down-regulated respectively (compared to controls) at 0

236   h, which was higher than at any of the other time points (Figure 2). Moreover, 76 and 120

237   proteins were statistically up-regulated in cells exposed to 55 and 100 PSU respectively at 0

238   h, indicating the initial acclimation strategy towards high salinity conditions. List of

239   differentially expressed proteins in *M. gaditana* in high salinity conditions (55 and 100 PSU)

240   are given in supplementary table S1.

241

242   **Figure 2.** Volcano plot showing significantly regulated proteins as green dots (p-value<0.05

243   and absolute log2(fold change) >1). Values on either side of the plot represent the number of

244   significantly up- and down-regulated proteins.

245

246   *GO-term enrichment reveals the path towards acclimation*

247

248   The disparity in the number of differentially expressed proteins at various time points and

249   different salinity levels suggests that *M. gaditana* employs a different strategy for acclimation

250   to salinities of 55 and 100 PSU. To understand the path towards acclimation, statistically

251   significant proteins at different time points were GO-enriched in Blast2GO using a filtering

252   cut-off of p-value<0.05 (Conesa et al., 2005). Interactive maps were created with the

253   significantly enriched biological process (BP) GO-terms (supplementary tables S2 and S3)

254   using REVIGO (Supek et al., 2011). Figure 3 shows the enriched biological process (BP)

255   GO-terms at different time points for cells exposed to 55 and 100 PSU, except for the 100

256   PSU cells at 24 h where the BP terms were not statistically enriched. Therefore, a molecular

257   function GO-term was used to describe the acclimation path. The interaction map of enriched

258   GO-terms clearly shows that *M. gaditana* exposed to 55 and 100 PSU use a distinct strategy

259   to overcome salinity, except at 1 h after the transfer to the elevated salinity, where the GO-

260   term for translation is enriched in both 55 and 100 PSU. The initial response to changes in

261   salinity levels was significantly enriched by the photosynthesis GO-term in cells in both 55

262   and 100 PSU. Similarly, proteomic analysis on a halotolerant alga *Dunaliella salina* revealed

263   the dynamics of expression of essential proteins involved in photosynthesis at high salinity

264   conditions (Wei et al., 2017).

265

266   Following our previous study on *M. gaditana* during the transfer to high-salinity conditions

267   (Karthikaichamy et al., 2018), GO-terms for carbohydrate metabolism were enriched after 72

268 h in 100 PSU. Relatively fewer GO-terms were enriched at 6 and 24 h after transfer, which is

269 a reflection of the number of differentially expressed proteins in cells growing at 55 and 100

270 PSU. However, a varied response to different saline conditions was evident across the time

271 points. Salinity- induced ROS accumulation in cells at 55 PSU was observed in our previous

272 study (Karthikaichamy et al., 2018) and correspondingly, the GO-terms for response to stress

273 (GO:0033554) and cellular oxidant detoxification (GO:0098869) were enriched at 6 and 24 h

274 respectively.

275

276 In contrast, GO-terms for energy-intensive translation (GO:0006412) was enriched at 1 and 6

277 h after transfer of cells to 100 PSU, suggesting a protein aided metabolic shift during the later

278 phases (72 h) of acclimation through carbohydrate metabolism (GO:0005975) and

279 homeostatic processes (GO:0042592). The enrichment of the carbohydrate metabolism

280 (GO:0005975) term during the later-phase of growth suggests carbon shunting towards lipid

281 accumulation, which is evident from our physiological (Karthikaichamy et al., 2018) and

282 transcriptomic studies on *M. gaditana* (un-published).

283

284 **Figure 3.** Interaction map of enriched GO-terms at different cultivation time points and

285 salinities in *M. gaditana*. Bubble color indicates the p-value; bubble size indicates the

286 frequency of the GO term.

287

288 *Salinity-responsive proteins (SRPs) identified by correspondence analysis*

289

290 Correspondence analysis (CA) was performed to find the association between different time

291 points and differentially expressed proteins in response to saline conditions (55 and 100

292 PSU). CA is a multivariate method that is used to reduce the dimensions of a complex dataset

293 into a low-dimensional space while preserving the information (Greenacre, 1984). CA has

294 been widely used in exploring the interactions between the genes and experimental

295 conditions (Fellenberg et al., 2001) or tissue types (Kishino & Waddell, 2000). The temporal

296 proteomic data was subjected to CA using '*ade4*' package in R (Dray & Dufour, 2007).

297

298 The data was spread out on a two-dimensional biplot, which accounted for 73.5% and 76.5%

299 of total inertia in cells exposed to 55 and 100 PSU respectively. In the biplot (figure 4), each

300 point represents a protein, and the arrows that originate from origin represent time points.

301 Proteins closer or along the arrow are up-regulated at that particular time point, and the

302 proteins that are on the opposite quadrant of the arrow represent the down-regulated protein

303 at that particular time point. Proteins are colored according to the contribution towards the

304 plot; the highest contribution indicates that the protein has been highly expressed at that

305 particular time point.

306

307 **Figure 4.** Correspondence analysis of differentially expressed proteins in *M. gaditana* at

308 different time points in 55 and 100 PSU. Biplot showing the association between time points

309 and differentially expressed proteins (numbers along the red arrow represent time points).

310

311 ***Top salinity-responsive proteins in cells grown in hypersaline conditions***

312

313 Top 10 proteins, based on their contribution values, for each salinity conditions, are shown in

314 figure 5. Three uncharacterized proteins were found to be among the top 10 contributors in

315 55 PSU (W7T9L0, W7TQA5, and W7T0M7) and two were found in cells exposed to 100

316 PSU (W7U7C1 and W7T797). The top contributor in cells exposed to 55 PSU was W7T3F9,

317 an esterase lipase thioesterase family protein, which was up-regulated at 0 h and 6 h. It has

318 been GO annotated with acylyglycerol lipase activity (GO:0047372) and medium-chain fatty

319 acid catabolic processes (GO:0051793). The gene product (at2g39420) of the protein

320 homolog in *Arabidopsis* (Q8RXN7_ARATH; E-value: 1.7e-65) was up-regulated (1.7

321 log2FC) in response to salt and heat stress. It also responds to drought stress and ozone

322 exposure (Abdeen et al., 2010; Suzuki et al., 2016). The esterase lipase thioesterase family

323 protein is widely involved in mobilizing oil reserves to provide carbon backbones for energy

324 sources during abiotic and heavy metal stress (Gao et al., 2010; Troncoso-Ponce et al., 2013).

325 The other early response protein is Nudix hydrolase 6-like protein (W7U4T1), which was up-

326 regulated ~64-fold at 0 h. The *Arabidopsis* Nudix hydrolases positively regulate salicylic

327 acid-mediated signaling and modulates defense responses against abiotic and biotic stress (Ge

328 & Xia, 2008; Jambunathan & Mahalingam, 2006).

329

330 Another protein whose abundance is highly modulated during the high saline condition (55

331 PSU) is quinoprotein amine dehydrogenase, beta chain (W7TI92). W7TI92 was up-regulated

332 ~3-fold at 1 and 24 h, and down-regulated up to 5.5-fold at other time points (0, 6 and 72 h).

333 The quinoprotein amine dehydrogenase, beta chain has a beta-propeller structure, which is

334 similar to the YVTN (Tyr-Val-Thr-Asn) repeat in the N-terminal of archaeal surface proteins.

335 The structural similarity to the archaeal surface proteins is of high significance because it

336 confers protection to the cell during extreme environmental conditions (Jing et al., 2002).

337 Cystathionine beta-synthase (W7TGG0) which catalyzes the formation of cystathione from

338 L-serine and L-homocysteine is variably expressed through-out the growth phase and the

339 highest expression observed at 1 h (~3x). The ortholog of W7TGG0 in rice plant

340 (OsCBSCBSPB4), when over-expressed, was found to be responsible for the increase in

341 biomass and delayed leaf senescence during extreme conditions (high saline, dehydration,

342 high temperature) (Kumar et al., 2018).

343

344 **Figure 5.** Heatmap of the top 10 differentially expressed proteins in *M. gaditana* based on the

345 contribution towards the dimensions in the biplot. Color scale indicate log_2FC value.

346 Significant levels of protein abundance are marked with an asterisk.

347

348 An ortholog of W7T0M7 (Uncharacterized protein (Fragment)) was up-regulated ~32-fold at

349 6 h, and a modest down-regulation and up-regulation were observed at 1, 24 h and 0, 72 h

350 respectively. The ortholog (A0A0A7CLB7) was found in a fungus *Thraustotheca clavate* (E-

351 value: 8.4e-9) is a secreted protein, belonging to RNase T2 family (IPR033697). Plant T2

352 RNases scavenge phosphate from ribonucleotides during senescence, and they also respond

353 to wounding or pathogen invasion (Deshpande & Shankar, 2002).

354 On the other hand, the top contributing protein in 100 PSU is an uncharacterized protein

355 (W7U7C1), which is predicted to be localized in chloroplast, mitochondria, and nucleus. The

356 protein is found in high abundance (~215x) during the initial phases (0 h) of growth in 100

357 PSU and at a modest up-regulation of up to 8x and 3X at 6 and 72 h respectively. The paralog

358 for W7U7C1 is an Hsp70-interacting protein (K8YSA6; E-value: 7.8e-133), which is

359 involved in Hsp70 stabilization in ADP (adenosine diphosphate) bound conformation

360 (Palleros et al., 1993). Hsp70-interacting protein (Hip), which is ubiquitously found in

361 animals and plants is involved in the regulation of Hsp70 assisted protein folding and import

362 (Boorstein et al., 1994; Boston et al., 1996; Miernyk, 1999). The strong up-regulation of

363 W7U7C1 during the initial stages of growth in 100 PSU signifies the importance of protein

364 folding upon stress-induced protein unfolding, thereby maintaining proteostasis during stress

365 conditions (Kim et al., 2013). One of the most abundant proteins expressed in response to the

366 high saline condition is glutamate decarboxylase (W7TVC7). It was strongly up-regulated by

367 1450-fold at 0 h, and then a modest up-regulation (up to 8x) was observed at other time

368 points. Plants produce γ-aminobutyrate by irreversible α-decarboxylation of glutamate by

369 glutamate decarboxylase. GABA acts as a natural defense against pests, and it is also

370   associated with stress response in tobacco and *Camellia sinensis* (tea) plants (McLean et al.,

371   2003; Mei et al., 2016).

372

373   Proteins involved in translation were variably affected at the highest saline conditions (100

374   PSU). For instance, ribosome recycling factor (W7TPY1) and 60s acidic ribosomal protein

375   (W7TV03) responded well to change in salinity levels. While the ribosome recycling factor

376   protein (W7TPY1), which is involved in translation and chloroplast biogenesis was up-

377   regulated up to 12-fold at 0 and 24 h, the 60 S acidic ribosomal protein (W7TV03), which

378   plays an essential part in elongation of proteins (Naganuma et al., 2010) was consistently

379   down-regulated (up to ~11x) at all time points, except at 1 h where a modest up-regulation

380   (~1.8) was observed. The availability of nitrogen could limit the translation of protein, and

381   therefore, plants have evolved a strategy to salvage nitrogen from an N rich source such as

382   ureides. Guanine deaminase (W7U1P4) is an enzyme involved in purine nucleotide

383   catabolism, in which guanine is converted into xanthine and then to allantoin in further steps

384   of catabolism, which is considered to be the first step of salvaging nitrogen from guanine.

385   Allantoin accumulation is linked with salt stress tolerance in *Arabidopsis thaliana* (Irani &

386   Todd, 2016; Lescano et al., 2016). Likewise, in our study, the levels of guanine deaminase

387   (W7U1P4) was up-regulated (up to ~8x) at 0, 24 and 72 h, which indicates a possible salvage

388   mechanism of nitrogen (N_2) by *M. gaditana* from purine nucleotides.

389

390   *Salinity induced proteome changes in cellular metabolism*

391

392   The proteome dynamics of *M. gaditana* was studied under salinity stress conditions. The

393   differentially expressed proteins at various time points were subjected to GO enrichment

394   analysis, which revealed a diverse response to various high salinity levels (55 and 100 PSU).

395   Similarly, we observed salinity induced dynamics in the transcriptome of *M. gaditana* (un-

396   published). In order to understand the fate of cellular metabolism at high saline conditions,

397   and to connect a line between physiology (Karthikaichamy et al., 2018) and transcriptome

398   (un-published) of salinity induced *M. gaditana*, the differentially expressed proteins were

399   analyzed for their biological significance in the following sections.

400

401

402

403

404   *Mitigation of ROS levels by rapid accumulation of stress response proteins*

405

406   Imbalance in light-harvesting and utilization in salinity stressed phototrophs could generate

407   ROS, which is deleterious to the cell (Miller et al., 2010). ROS scavenging and homeostasis

408   are critical for the shift in metabolism after salinity stress is imposed. Several proteins

409   involved in oxidative stress responses were up-regulated at the high saline conditions (55 and

410   100 PSU) (supplementary table S4). Overall, the fold change of proteins involved in

411   oxidative stress responses (such as superoxide dismutase, catalase, and ferredoxin) was up-

412   regulated (up to ~8x) at 1 h in 55 PSU and 1 h and 72 h in 100 PSU, except a few proteins

413   such as superoxide dismutase (W7TM73) and ferredoxins (W7TM71). The other superoxide

414   dismutases (W7T7U7, W7TGS6, W7U428) were significantly expressed (up-regulation up to

415   ~5x at 1 and 72 h) in 100 PSU. Several glutaredoxins, glutathione transferase (W7TNY9;

416   W7U7N8), ascorbate peroxidase (W7U0U0) and thioredoxins were significantly altered in

417   100 PSU. ROS accumulation is a signature of stress response in plants and microalgae.

418   Comparable with the metabolic changes, the salinity induced ROS accumulation triggered a

419   set of ROS scavenging proteins such as SOD, CAT, glutathione peroxidase, ferredoxin in *M.*

420   *gaditana*. These proteins are involved in maintaining the redox status in higher plants (Chen

421   et al., 2009; Kav et al., 2004; Sugimoto & Takeda, 2009). Likewise, the SOD level was

422   increased in the halotolerant alga *Dunaliella salina* (Wei et al., 2017) and in a salinity

423   tolerant strain of *Chlamydomonas reinhardtii* (Sithtisarn et al., 2017). Anti-oxidant proteins

424   help to maintain the ROS level inside the cell, and accumulation of such proteins have been

425   found in salinity treated plants (Miller et al., 2010). Mitigation of ROS levels by rapid

426   accumulation of scavenging enzymes could be an initial step towards acclimatization of *M.*

427   *gaditana* to saline conditions. The expression profiles of the significantly altered oxidative

428   stress response proteins are shown in Figure 6.

429

430   **Figure 6.** Expression levels of proteins involved in oxidative stress responses in *M. gaditana*

431   (grey lines). The red line indicates the mean expression values of the significantly altered

432   proteins. Error bars indicate standard deviations.

433

434

435

436

437

438   *Hypersalinity induces up-regulation of osmolyte biosynthesis proteins*

439

440   Osmolyte production is one of the primary responses of microalgae towards an increase in

441   salinity (Flowers & Colmer, 2008). The osmolytes are highly soluble, low-molecular-weight

442   molecules primarily accumulated in the cytoplasm. They range from simple carbohydrates

443   such as trehalose, xylose and sucrose to amino-acids such as glycine, proline, or amino-acid

444   derivatives such as ornithine (Flowers et al., 1977; Wyn Jones, 1977). The expression profiles

445   of the proteins involved in osmolyte biosynthesis are shown in Figure 7. In our study, we

446   observed an increase in protein expression levels of key enzymes involved in trehalose,

447   glycine betaine, and glycerol biosynthetic pathways (supplementary table S5).

448

449   **Figure 7.** Expression levels of key enzymes involved in osmolyte biosynthetic pathway in *M.*

450   *gaditana* under high saline conditions (grey lines). Blue line indicates the mean expression

451   values of the significantly altered proteins. Error bars indicate standard deviations.

452

453   Trehalose is a non-reducing disaccharide formed by two glucose molecules linked by alpha-

454   1,1-bond. Accumulation of trehalose during stress conditions such as drought, salt, heat,

455   desiccation and freeze stress has been reported across various microorganisms, including

456   plants and microalgae (Goddijn & van Dun, 1999; Hershkovitz et al., 1991; Hirth et al.,

457   2017). Moreover, trehalose confers tolerance against abiotic stresses in rice (Garg et al.,

458   2002) and it is found to be essential for carbohydrate utilization in *Arabidopsis thaliana*

459   (Schluepmann et al., 2003). The levels of trehalose-6-phosphate synthase (W7TQF4),

460   trehalose phosphate synthase (W7TD30 and W7TBU1) and trehalose 6-phosphate

461   phosphatase (W7TFM0) were significantly altered only in 100 PSU, and they were up-

462   regulated (up to ~16x) during the later phases of growth (72 h).

463

464   The enzyme that prime trehalose biosynthetic pathway, UDP-glucose pyrophosphorylase 2

465   (W7TFM0), by providing UDP-glucose as substrate was significantly altered only in 100

466   PSU. The protein abundance increased (~19x) at 72 h in100 PSU. Interestingly, the

467   expression pattern of UDP-glucose pyrophosphorylase 2 is similar to the trehalose

468   biosynthetic proteins, implying the importance of the UDP-glucose pool for trehalose

469   biosynthesis during high salinity conditions (Figure 8).

470

471 **Figure 8.** Salinity induced temporal dynamics of proteins involved in trehalose biosynthesis

472 in *M. gaditana*. Significant levels of protein abundance are marked with an asterisk.

473

474 Other proteins involved in compatible solute biosynthesis include choline dehydrogenase

475 (W7TDH7, W7TN63), which catalyzes the first step in glycine betaine biosynthesis through

476 betaine aldehyde, and which was significantly altered only in 100 PSU. The maximum up-

477 regulation (up to ~4x) occurred at 1 and 72 h post salinity stress induction. Similar dynamics

478 in betaine biosynthesis was observed in our earlier transcriptomic study. The assimilation of

479 CO_2 into glycine betaine during saline conditions was observed in *A. halophytica* (Takabe et

480 al., 1988). Also, exogenous addition of glycine betaine to the growth medium of *A. thaliana*

481 conferred tolerance to abiotic stresses (Hibino et al., 2002).

482

483 A key enzyme in glycerol (compatible solute) biosynthesis, glycerol-3-phosphate

484 dehydrogenase (W7TIR6) is significantly up-regulated (up to ~8x) during later phases of

485 growth (72 h) in 100 PSU. GPDH is located in the mitochondrial inner-membrane space or

486 cytosol and catalyzes the reduction of dihydroxyacetone phosphate into glycerol-3-

487 phosphate. It also links carbohydrate and lipid metabolism by providing glycerol backbones

488 for triacylglycerol (TAG) synthesis (Ou et al., 2006). Salinity induced *Dunaliella salina*

489 accumulates a significant amount of glycerol, which is comparable with GPDH activity (Wei

490 et al., 2017).

491

492 *Proteins involved in fatty acid metabolism*

493

494 Fatty acids are energy reservoirs of the cell that can be used during nutrient limitation

495 conditions or any adverse environmental conditions (Mata-Pérez et al., 2016). Cell

496 membranes play a vital role in sensing the stress conditions and initiate the acclimatization

497 reactions. The fluidity of plant and algal cell membrane can be altered by changing the fatty

498 acid composition of the membrane. This phenomenon is beneficial for the organism to adapt

499 to the stress conditions (Mikami & Murata, 2003; Wada et al., 1994). Salt induced changes in

500 neutral-lipid content in *M. gaditana* have already been reported in our previous study

501 (Karthikaichamy et al., 2018). Comparable levels of transcript induction of lipid biosynthetic

502 genes were observed in *M. gaditana*. The expression profiles of significantly altered proteins

503 during hypersaline conditions are shown in Figure 9.

504

505   In line with our physiological analysis, we have identified proteins involved in lipid and fatty

506   acid metabolism (supplementary table S6). Four proteins involved in lipid metabolism were

507   statistically altered in 55 PSU, among which are an esterase lipase thioesterase family protein

508   (W7T3F9), fatty acid desaturase type 2 (W7UCJ8) and 3-oxoacyl-(Acyl-carrier-protein)

509   reductase (W7U8F0), which were up-regulated more than 2-fold. 3-oxoacyl-(Acyl-carrier-

510   protein) reductase (W7U8F0) is involved in the NADPH-dependent reduction of beta-

511   ketoacyl-ACP substrates in the elongation cycle of fatty acid biosynthesis (Lai & Cronan,

512   2004). An instantaneous response to salinity stress was observed with an abundance of

513   esterase lipase thioesterase family protein (W7T3F9) during the early phases of growth (0 h)

514   in both saline conditions (55 and 100 PSU). The implications of esterase lipase thioesterase

515   family protein during the alleviation of salinity stress have been discussed earlier.

516

517   **Figure 9.** Expression levels of proteins involved in fatty acid metabolism (grey lines) in *M.*

518   *gaditana*. Orange line indicates the mean expression values of the significantly altered

519   proteins. Error bars indicate standard deviations.

520

521   In 100 PSU, proteins involved in lipid biosynthesis such as long-chain acyl-CoA synthetase

522   (W7TGG5), diacylglycerol acyltransferase family protein (W7U9S5),

523   monogalactosyldiacylglycerol synthase, long-chain acyl-synthetase 7 (W7TAS6) and several

524   lipases were statistically up-regulated (up to ~8x) during the later phases of growth (72 h).

525   The significant up-regulation of the proteins involved in lipid metabolism is consistent with

526   our previous physiological studies on *M. gaditana* (Karthikaichamy et al., 2018). Long-chain

527   acyl-CoA synthetase (LACS) is indispensable for biosynthesis of fatty acid derived

528   molecules. In germinating *Arabidopsis thaliana* seedlings, LACS activate the free fatty acids

529   to the β-oxidation pathway for the removal of two-carbon molecule in the form of acetyl-

530   CoA (Fulda et al., 2004). The acetyl-CoA thus formed enters the TCA cycle for the

531   production of cellular energy (Gerhardt, 1992). It is therefore evident that *M. gaditana*

532   prefers β-oxidation of accumulated fatty acid for sustaining the energy requirements during

533   high saline conditions. Though the cell primarily focusses on β-oxidation of lipids, a

534   comparable up-regulation (~8x) of diacylglycerol acyltransferase family protein (W7U9S5),

535   which is involved in triacylglycerol (TAG) synthesis is observed during the later phases (72

536   h) in 100 PSU. This phenomenon could account for a steady, but a not sharp, increase in

537   neutral lipid content in *M. gaditana* (Karthikaichamy et al., 2018). *Arabidopsis thaliana*

538   diacylglycerol acyltransferase mutants were sensitive to several stress conditions, implying a

539   possible role in stress mitigation (Lu & Hills, 2002). Lipolytic protein g-d-s-l family protein

540   (W7TVB2) was significantly up-regulated (up to ~64x) at later-phases of growth in 100 PSU.

541   The lipolytic protein g-d-s-l family have been studied in plant species such as *Arabidopsis*,

542   rice, and maize, and it has been associated with plant development and defense responses

543   (Brick et al., 1995). Overexpression of GDSL☐motif lipase increases tolerance in transgenic

544   *A. thaliana* (Naranjo et al., 2006).

545

546   ***Oxidative pentose-phosphate pathway redirects the carbon flux towards fatty acid***

547   ***biosynthesis under high saline conditions***

548

549   The primary source of NADPH for desaturation and elongation reactions of polyunsaturated

550   fatty acid (PUFA) is malic enzyme (ME). However, in our study, malic enzyme was not

551   significantly up-regulated. Various studies have established a connection between malic

552   enzyme levels and neutral lipid accumulation (Zhu et al., 2018). Also, the transcript

553   abundance of malic enzyme was down-regulated in our transcriptomic analysis on *M.*

554   *gaditana* (un-published). Corresponding to the previous hypothesis established on the use of

555   oxidative pentose phosphate pathway as an alternative source to TCA cycle for reducing

556   equivalents, one of the key proteins (glucose-6-phosphate 1-dehydrogenase) involved in the

557   pentose phosphate pathway was significantly up-regulated (~16x) during the later phases of

558   growth (24 and 72 h) in 100 PSU (supplementary table S6). Glucose-6-phosphate 1-

559   dehydrogenase (W7TZ08), catalyzes the rate-limiting step of the oxidative pentose-phosphate

560   pathway (PPP) and provides reducing equivalents (NADPH) and pentose phosphates for fatty

561   acid and nucleic acid biosynthesis respectively (Wang et al., 2014). Thus, *M. gaditana*

562   establishes lipid homeostasis via simultaneous activation of β-oxidation and TAG

563   biosynthesis proteins during high saline conditions. Another rate-limiting enzyme in PPP, 6-

564   phosphogluconate dehydrogenase (PGDH), decarboxylating (W7TN92) was significantly up-

565   regulated (up to ~16x) during the later phases of growth (72 h), suggesting that PPP is

566   enhanced during high saline condition in *M. gaditana*. PPP is a key pathway in carbohydrate

567   metabolism in plants and links other biosynthetic processes such as lipid, nucleotide, sugar

568   and aromatic amino acid biosynthesis by providing reducing equivalents (NADPH) and

569   precursor molecules. The increase in abundance of the critical proteins involved in reductive

570   pentose phosphate pathway complements the increased glycerol biosynthetic protein

571   abundance. PPP aids in glycerol accumulation by replenishing the carbon intermediates

572   (ribulose-5-P). A similar trend was observed in higher plants (rice and wheat), where salt

573   stress induced the expression of PGDH and GPDH (Krishnaraj & Thorpe, 1996). Our results

574   suggest that PPP helps in redirecting the carbon flux towards fatty acid biosynthesis under

575   high saline conditions in *M. gaditana*.

576

577   ***Photosynthesis and redox energy metabolism***

578

579   Salinity stress is often linked with the reduction in photosynthetic efficiency (Karthikaichamy

580   et al., 2018), primarily due to damage caused by ROS to light-harvesting complexes (LHCs)

581   and oxygen evolution factor proteins of PSI (Subramanyam et al., 2010). In other instances,

582   cessation of PSII activity was observed in *Dunaliella tertiolecta* and *Nannochloropsis* sp.

583   under high salinity conditions (Gilmour et al., 1985; Martínez-Roldán et al., 2014). A

584   comprehensive list of differentially expressed proteins involved in photosynthesis is provided

585   in supplementary table S7.

586

587   Porphobilinogen deaminase (W7UC09), which catalyzes the primary step of chlorophyll

588   biosynthesis (Roberts et al., 2013) was down-regulated (up to ~4x) throughout the growth

589   phase (significant down-regulation at 72 h), except at 1 h. The reduction in the abundance of

590   a critical chlorophyll biosynthetic protein was reflected in the reduction of chlorophyll

591   content in salinity stressed *M. gaditana* (Karthikaichamy et al., 2018). Reduction in

592   chlorophyll biosynthetic protein levels was also observed in salinity stressed *D. salina* (Wei

593   et al., 2017). Reduction in chlorophyll levels during stress conditions can be associated either

594   with the cessation of biosynthesis or dilution by cellular growth. The latter was reported in *C.*

595   *reinhardtii* (Juergens et al., 2015), while the former phenomenon was observed in our case.

596   Interestingly, various proteins associated with PSII (oxygen evolving complex, D1 protein,

597   D3 protein, PsbP, cytochrome *c*, ferredoxin-NADP reductase, PSII 47 kDa protein and light-

598   harvesting complex proteins) and PSI (apoprotein A1, A2 and PSI reaction center proteins)

599   were up-regulated (up to ~8x) during the initial phases of growth (0 and 1 h) in both the

600   salinity levels (55 and 100 PSU). A few PSII and PSI proteins were also up-regulated (up to

601   ~8x) during the later phases of growth in 100 PSU. The inner antenna PSII 47 kDa protein is

602   conserved and is essential for assembling PSII in algae and higher plants (Summer et al.,

603   1997). Cytochrome *b*559 subunit alpha (K9ZWI6) and cytochrome *b*559 subunit beta

604   (K9ZVG9) protects PSII from photo-inhibition by enhancing cyclic electron transport (CET)

605   in *Chlamydomonas reinhardtii* (Burda et al., 2003) is significantly up-regulated (up to ~2x)

606   in 55 PSU. Enhanced photosynthesis and energy metabolism during high salinity conditions

607   has been linked with increased CO_2 fixation and glycerol biosynthesis in *Dunaliella salina*

608   (Liska et al., 2004). The expression profiles of the proteins involved in photosynthesis are

609   shown in Figure 10.

610

611   **Figure 10.** Expression levels of proteins involved in photosynthesis (grey lines) in *M.*

612   *gaditana*. Green line indicates the mean expression values of the significantly altered

613   proteins. Error bars indicate standard deviations.

614

615   Several light-harvesting complexes of PSII (LHCII) were found to be up-regulated (up to

616   ~5x) in 55 (1 h) and 100 PSU (1 and 72 h). Enhancement of LHCII proteins was also

617   observed upon palmella formation in salinity induced *Dunaliella salina*. During stress

618   conditions, regulation of redox homeostasis in the chloroplast is enhanced by reversible

619   phosphorylation of LHCII proteins (Grieco et al., 2012). In photosynthetic micro-organisms

620   and higher plants, carbonic anhydrases (CA) are responsible for converting HCO_3^- to CO_2,

621   which is then fixed by ribulose 1,5-bisphosphate carboxylase/oxygenase (Espie & Kimber,

622   2011). There are multiple forms of carbonic anhydrases involved in a host of reactions in the

623   cell, and an external carbonic anhydrase that plays a role in CO_2 concentrating mechanisms.

624   Up-regulation (up to ~8x) of carbonic anhydrase (W7TZZ4) was observed only in 100 PSU

625   at 72 h. The protein abundance of the target of carbon concentrating mechanism (CCM),

626   ribulose bisphosphate carboxylase (K9ZV74, K9ZWI1) was not significant. Thus, the protein

627   abundance of carbonic anhydrase could be beneficial for the cell during adverse conditions

628   rather than to invest in an energetic input for Rubisco biosynthesis (Young & Hopkinson,

629   2017). Upregulation of external CA is induced by hypersaline conditions in *Dunaliaella*

630   *salina* as dissolved CO_2 levels are lower with increasing salinity. While, Rubisco activity was

631   inhibited in response to high saline conditions in *D. salina* (Booth and Beardall, 1991).

632

633   **Conclusions**

634

635   The current study aimed to understand the temporal proteome dynamics during

636   acclimatization to high saline conditions (55 and 100 PSU). The timepoints and salinity

637   conditions were chosen based on our previous physiological studies on *M. gaditana* at high

638   saline conditions. Significantly altered proteins at each time point were enriched with GO-

639   terms using fisher exact analysis. GO-enrichment revealed the acclimatization path of *M.*

640   *gaditana* towards hypersaline conditions. Salinity level of 100 PSU had significant impact on

641   the proteome of *M. gaditana*. Correspondence analysis revealed the salinity-responsive

642   proteins and their possible metabolic implications was analyzed. Proteins involved in lipid

643   and osmolyte biosynthesis was observed to be up-regulated at high salinity conditions, which

644   could be one of the possible strategies of *M. gaditana* in acclimatizing to high salinity

645   conditions. Osmolyte helps in retaining the cellular morphology by acting as regulators of

646   turgor pressure. Lipid biosynthetic proteins were upregulated at high salt conditions, so as the

647   proteins involved in fatty acid oxidation. The oxidized product could serve as a precursor for

648   various biosynthetic pathways. Protein levels and the physiology of *M. gaditana* at high salt

649   conditions correspond to each other. This study provides a comprehensive overview of the

650   temporal proteome level changes in *M. gaditana* at high salt conditions. A probable

651   acclimatization strategy of *M. gaditana* during high-saline conditions was discussed,

652   emphasizing on osmolyte, lipid and photosynthesis and redox energy metabolism pathways.

653   The proteome analysis suggests a possible route towards acclimatization and a few potential

654   target proteins for strain improvement.

655

656   **Acknowledgements**

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673   **References**

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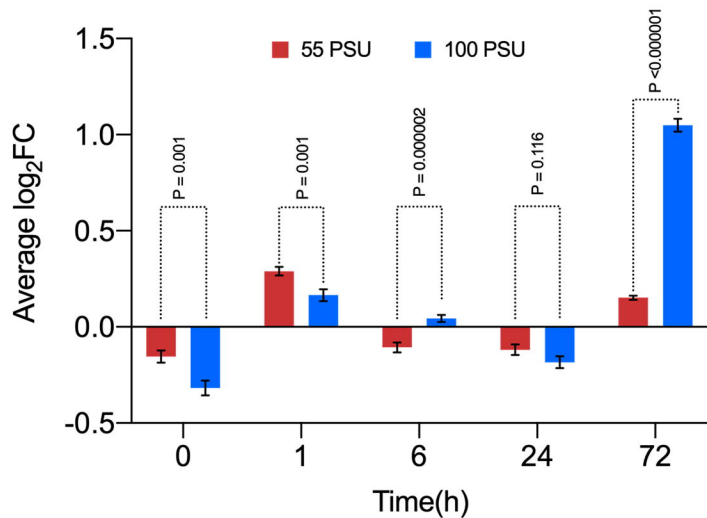897   database and its related tools. *Nucleic acids research*, **44**(D1), D447-D456.

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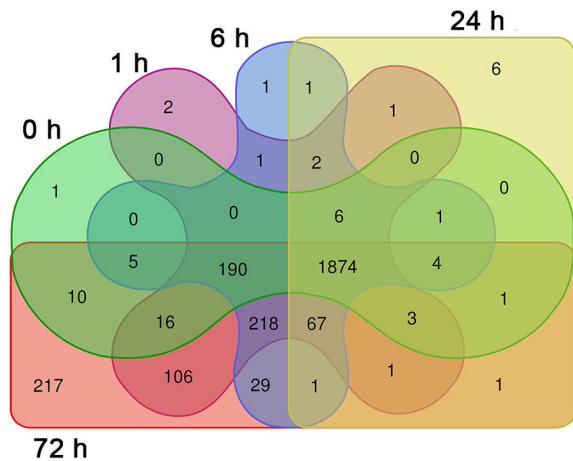
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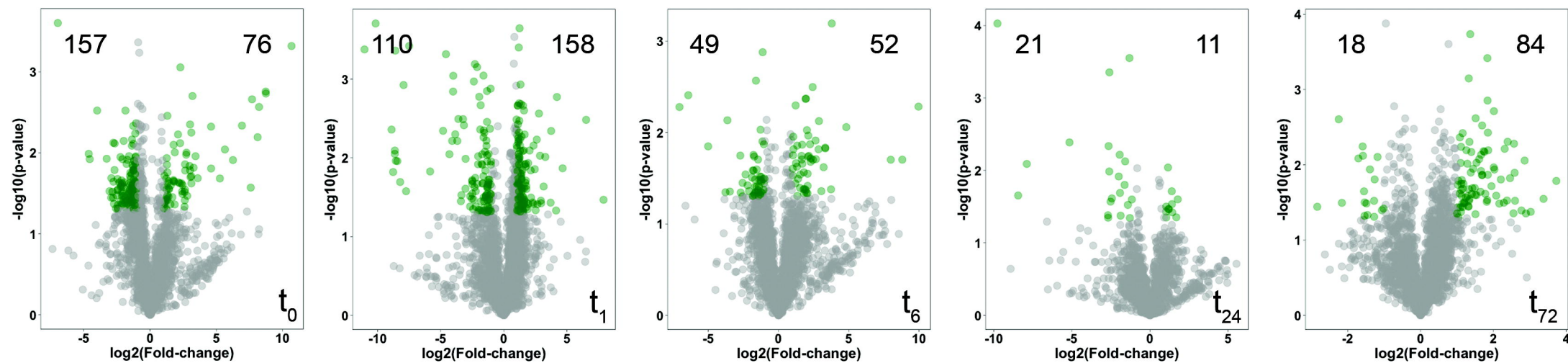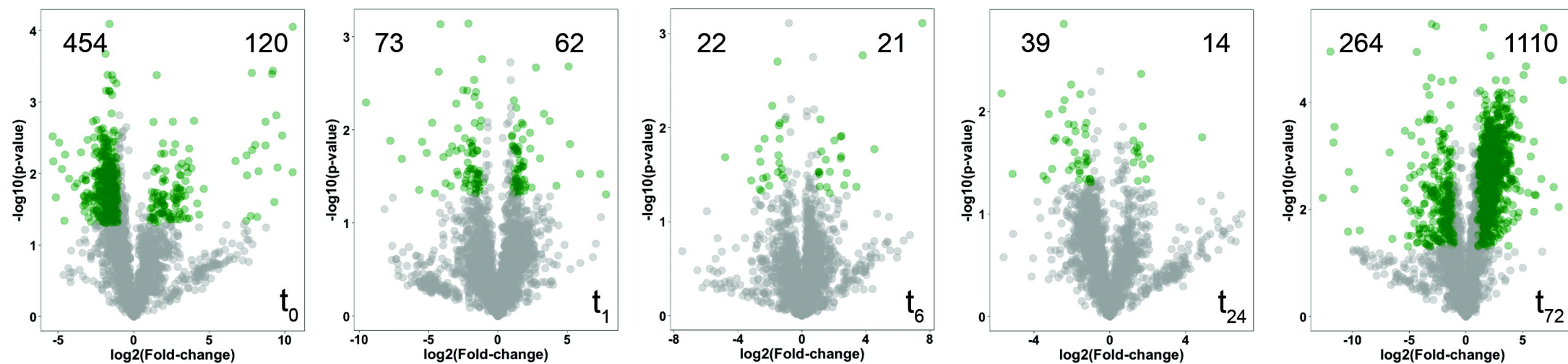918   *Phaeodactylum tricornutum. Frontiers in Plant Science*, **9**(826).
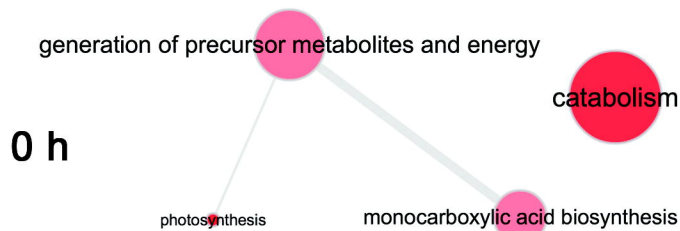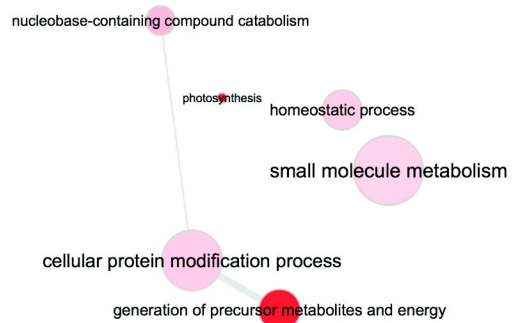
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A

B

55PSU vs Control

100PSU vs Control

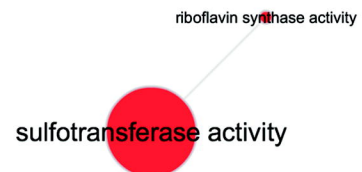|	55 PSU	100 PSU
0 h	generation of precursor metabolites and energy; catabolism; photosynthesis; monocarboxylic acid biosynthesis	nucleobase-containing compound catabolism; photosynthesis; homeostatic process; small molecule metabolism; cellular protein modification process; generation of precursor metabolites and energy
1 h	generation of precursor metabolites and energy; photosynthesis; translation; ribonucleoprotein complex assembly; cellular protein modification process	translation; ribosome biogenesis; peptidyl-proline hydroxylation
6 h	cell differentiation; reproduction; response to stress	translation
24 h	cellular oxidant detoxification	riboflavin synthase activity; sulfotransferase activity
72 h	DNA metabolism; pathogenesis; cell adhesion; cytoskeleton organization; peptidyl-proline hydroxylation; translational initiation	transmembrane transport; carbohydrate metabolism; homeostatic process; mitochondrion organization
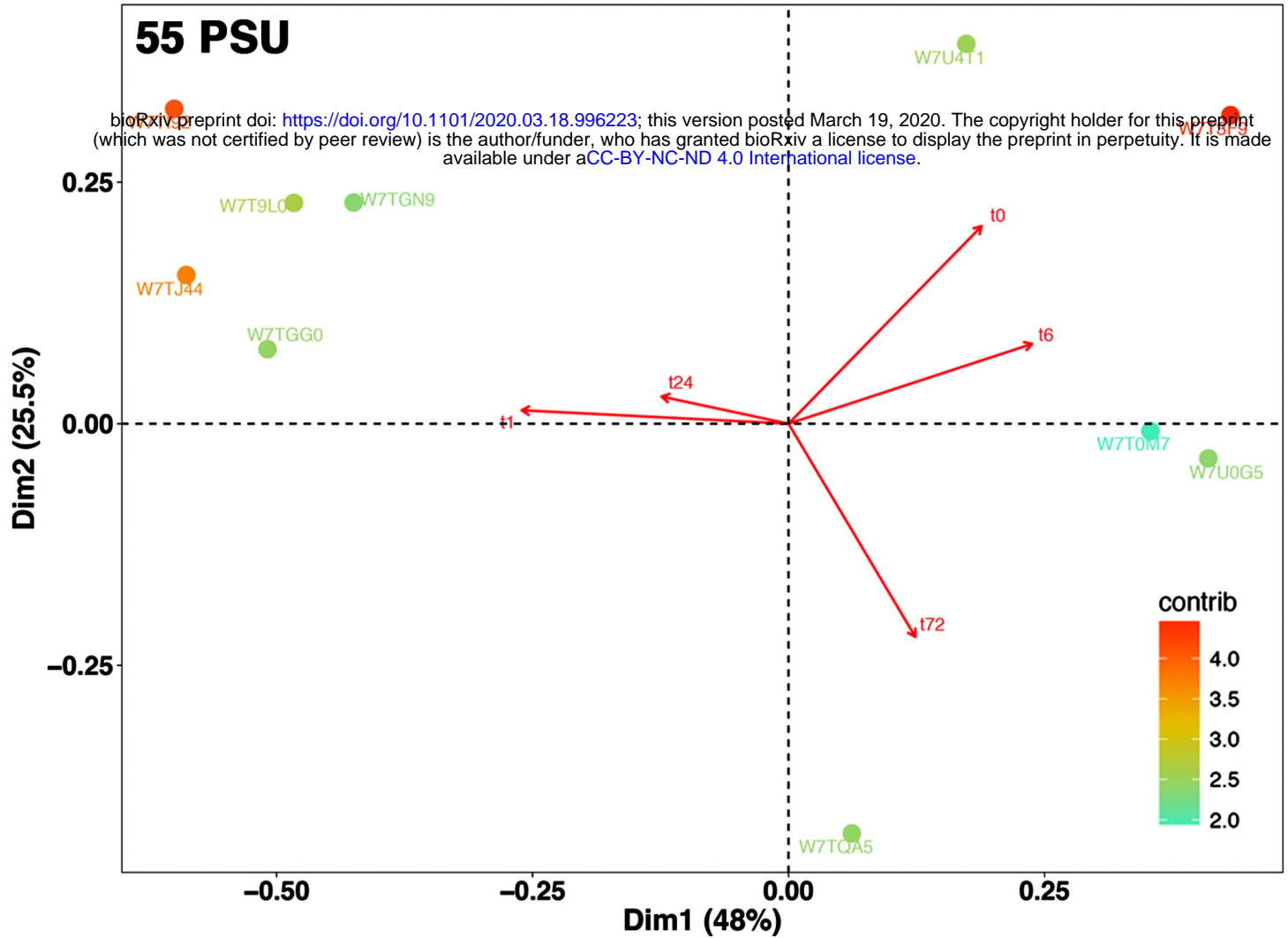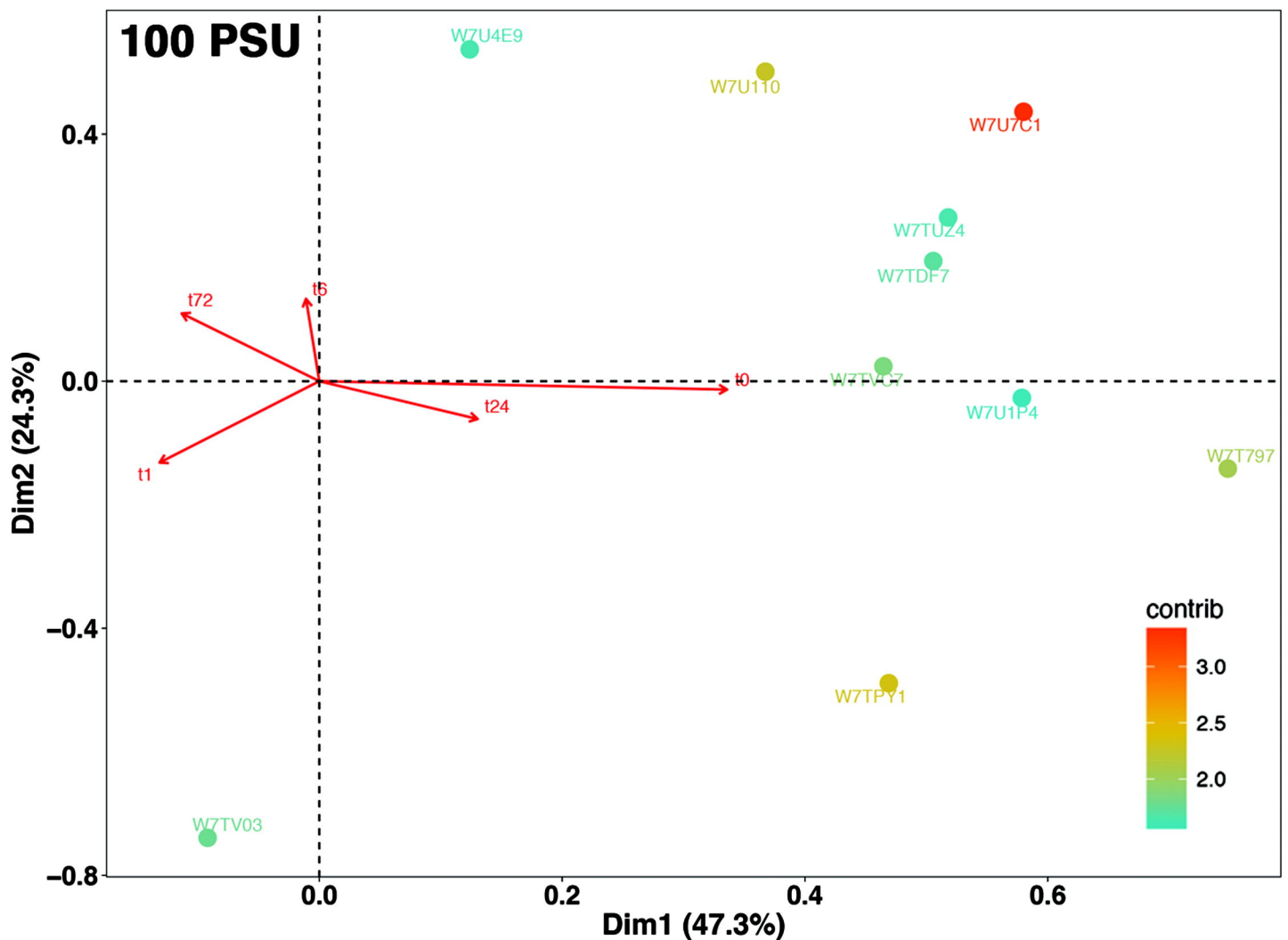
55 PSU

Time (h) 0	1	6	24	72	UniProtIds	Protein name	Contribution
*			*		W7T3F9	Esterase lipase thioesterase family protein	4.47
*				*	W7TI92	Quinoprotein amine dehydrogenase, beta chain	4.10
				*	W7TJ44	Uncharacterized protein	3.72
		*		*	W7T9L0	Uncharacterized protein	2.65
	*				W7U4T1	Nudix hydrolase 6-like protein	2.52
*	*			*	W7TGG0	Cystathionine beta-synthase	2.45
				*	W7TQA5	Uncharacterized protein	2.45
				*	W7U0G5	Trna (Cytosine-c)-methyltransferase	2.41
				*	W7TGN9	Zinc metalloendopeptidase	2.38
*				*	W7T0M7	Uncharacterized protein (Fragment)	1.91

100 PSU

Time (h) 0	1	6	24	72	UniProtIds	Protein name	Contribution
*				*	W7U7C1	Uncharacterized protein	3.34
				*	W7TPY1	Ribosome recycling factor	2.33
		*			W7U110	Histone H5	2.25
	*				W7T797	Uncharacterized protein	2.05
*				*	W7TVC7	Glutamate decarboxylase	1.84
*				*	W7TV03	60s acidic ribosomal protein	1.77
	*				W7TDF7	Lysyl-trna synthetase	1.70
			*	*	W7TUZ4	Uncharacterized protein	1.63
			*	*	W7U4E9	Two-component response regulator arr12	1.62
*				*	W7U1P4	Guanine deaminase	1.58

$\log_2 FC$

-10.50   0.00   10.50

55 PSU

100 PSU

Glucose 6-phosphate

+

UDP-glucose

Trehalose-6-phosphate synthase

Trehalose 6-phosphate

UDP-glucose pyrophosphorylase 2

Trehalose 6-phosphate phosphatase

Glucose 1-phosphate

+

UTP

Trehalose

Time (h)
0   72
100 PSU

log$_2$FC

-4 -2 0 2 4