Isolation of salt responsive genes and characterization of salt responsive mechanism in the marine diatom *Phaeodactylum tricornutum*

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Summary

Physiological and biochemical responses of the marine diatom *Phaeodactylum tricornutum* to various salt concentrations were investigated. Cells of the marine diatom *P. tricornutum* grown in f/2 enriched artificial seawater (F2ASW) containing 0.5 M NaCl were transferred to manipulated [NaCl] and cultured at 20 ºC for 2 weeks under continuous illumination. Cells were grown well under 0.1 M to 1.0 M NaCl. A partial deprivation of Cl^- by replacement of NaCl with Na_2SO_4 under constant [Na^+] of 1.0 M also exhibited little inhibitory effect on growth whereas the addition of 0.2 M LiCl, 0.2 M KCl, 1.0 M Sorbitol, or 0.9 M Cholinechloride to the F2ASW containing 0.1 M NaCl drastically inhibited the growth. These probable inhibitory effects with Cl^-, osmotic and cationic stresses were reduced by the addition of Na_2SO_4 in a concentration dependent manner. Under above-described stressed conditions, activities of the linear electron transport and PSII were measured with oxygen evolution rate respectively with and without added 2 mM 1,4-*p*-benzoqinon. All these activities were stimulated upon the addition of 0.45 M Na_2SO_4 to the modified F2ASW containing 0.1 M NaCl, whereas these were inhibited upon the addition of 0.9 M cholinechloride. The addition of 0.2 M LiCl, 0.2 M KCl, or 1.0 M sorbitol exhibited little effect on photosystem. These observations suggest a possible occurrence of novel halophilic systems in marine photoautotrophs.

Furthermore, Na^+ and Cl^- fluxes were monitored under several ambient [NaCl] using ion specific fluorescence indicators. At salt concentration below the average of seawater (0.5 M), both ions accumulated in periplastidal compartment, an intermembrane space of four-layered chloroplast envelope of secondary endcytobiotes. 360 µM ouabain and 100µM EIPA, inhibitors respectably for Na^+/K^+ ATPase and Na^+/H^+ antiporter, completely abolished Na^+ influx and dissipated periplastidal Cl^- accumulation at 0.5 M NaCl. These observations indicate that the periplastidal compartment might play a crucial role during acclimation of cells to broad range [NaCl], and that these ion fluxes are driven by Na^+/K^+ ATPase and/or Na^+/H^+ antiporter.