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Research paper

Transcriptional and physiological responses to inorganic nutrition in a tropical Pacific strain of *Alexandrium minutum*: Implications for nutrient uptakes and assimilation

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ABSTRACT

The marine dinoflagellate *Alexandrium minutum* is known to produce saxitoxins that cause paralytic shellfish poisoning in human worldwide through consumption of the contaminated shellfish mollusks. Despite numerous studies on the growth physiology and saxitoxin production of this species, the knowledge on the molecular basis of nutrient uptakes in relation to toxin production in this species is limited. In this study, relative expressions of the high-affinity transporter genes of nitrate, ammonium, and phosphate (*AmNrt2*, *AmAmt1* and *AmPiPT1*) and the assimilation genes, nitrate reductase (*AmNas*), glutamine synthase (*AmGSIII*) and carbamoyl phosphate synthase (*AmCPSII*) from *A. minutum* were studied in batch clonal culture condition with two nitrogen sources (nitrate: NO_3^- or ammonium: NH_4^+) under different N:P ratios (high-P: N:P of 14 and 16, and low-P: N:P of 155). The expression of *AmAmt1* was suppressed in excess NH_4^+-grown condition but was not observed in *AmNrt2* and *AmNas*. Expressions of *AmAmt1*, *AmNrt2*, *AmNas*, *AmGSIII*, *AmCPSII,* and *AmPiPT1* were high in P-deficient condition, showing that *A. minutum* is likely to take up nutrients for growth under P-stress condition. Conversely, relative expression of *AmCPSII* was incongruent with cell growth, but was well correlated with toxin quota, suggesting that the gene might involve in arginine metabolism and related toxin production pathway. The expression of *AmGSIII* is found coincided with higher toxin production and is believed to involve in mechanism to detoxify the cells from excess ammonium stress. The gene regulation observed in this study has provided better insights into the ecophysiology of *A. minutum* in relation to its adaptive strategies in unfavorable environments.

1. Introduction

Nitrogen (N) and phosphorus (P) are essential macronutrients in marine phytoplankton. They are important components in cell metabolism that involved in the nucleic acid synthesis, protein synthesis, phospholipids, photosynthesis, respiration, and energy transfer in adenosine triphosphate (ATP) form (Geider and La Roche, 2002; Cooper, 2000; Paytan and McLaughlin, 2007; Collos and Berges, 2003). In natural seawater, the N-based nutrients exist in the form of dissolved organic nitrogen (DON) (e.g., urea, free amino acids and protein) or dissolved inorganic nitrogen (DIN) (e.g., nitrate, nitrite, and ammonium) (Antia et al., 1991). DIN has been considered as a major nutrient form that sustains phytoplankton growth (Antia et al., 1991), but some dinoflagellates are capable of utilizing DON (Collos et al., 2004; Glibert et al., 2006). Likewise, the P-based nutrients exist in two forms, dissolved inorganic phosphorus (DIP) (orthophosphate) and dissolved organic phosphorus (DOP) (e.g., phosphoesters and phosphonates) (Young and Ingall, 2010). DIP is preferred by most phytoplankton due to the fact that it can be directly taken up and assimilated by the cells, whereas the process of converting DOP to DIP required extra cell energy (Falkowski and Raven, 2007). Different phytoplankton species groups prefer specific nutrient regimes, with varying nutrient

Abbreviations: Amt1, ammonium transporter; CPS, carbamoyl phosphate synthase; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; GS, glutamine synthase; HABs, Harmful Algal Blooms; Nas, nitrate reductase; Nir, nitrite reductase; NH_4^+, ammonia; NO_3^-, nitrate; Nrt2, nitrate transporter; N, nitrogen; P, phosphorus; PiPT1, phosphate transporter; PST, paralytic shellfish toxin; STX, saxitoxin

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