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Physiological and transcriptional responses to inorganic nutrition in a tropical Pacific strain of *Alexandrium minutum*: Implications for the saxitoxin genes and toxin production

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ABSTRACT

Saxitoxins (STXs) constitute a family of potent sodium channel blocking toxins, causative agents of paralytic shellfish poisoning (PSP), and are produced by several species of marine dinoflagellates and cyanobacteria. Two STX-core genes, *sxtA* and *sxtG*, have been well elucidated in *Alexandrium* but the expression of these genes under various nutritional modes in tropical species remains unclear. This study investigates the physiological responses of a tropical Pacific strain of *Alexandrium minutum* growing with nitrate or ammonium, and with various nitrogen to phosphorus (N:P) supply ratios. The transcriptional responses of the *sxt* genes were observed. Likewise, a putative *sxtI* encoding *O*-carbamoyltransferase (herein designated as *AmsxtI*) was recovered from the transcriptomic data, and its expression was investigated. The results revealed that the cellular toxin quota (Q_t) was higher in P-depleted, nitrate-grown cultures. With cultures at similar N:P (<16), cells grown with excess ammonium showed a higher Q_t than those grown with nitrate. *sxtA1* was not expressed under any culture conditions, suggesting that this gene might not be involved in STX biosynthesis by this strain. Conversely, *sxtA4* and *sxtG* showed positive correlations with Q_t, and were up-regulated in P-depleted, nitrate-grown cultures and with excess ambient ammonium. On the other hand, *AmsxtI* was expressed only when induced by P-depletion, suggesting that this gene may play an important role in P-recycling metabolism, while simultaneously enhancing toxin production.

1. Introduction

Saxitoxin (STX) is a tricyclic perhydropurine alkaloid that includes a family of naturally occurring neurotoxins. This alkaloid undergoes several natural substitutions at various structural positions, resulting in at least 57 known derivatives (Oshima, 1995; Onodera et al., 1997; Llewellyn et al., 2004; Lim et al., 2007a; Wiese et al., 2010). The toxin is a causative agent of paralytic shellfish poisoning (PSP) in humans, which has tremendous socio-economic impacts in affected countries (Anderson et al., 1996; Hoagland and Scatasta, 2006; Usup et al., 2012). The PSP-associated toxins (PSTs) are produced by several species of marine dinoflagellates and prokaryotic cyanobacteria. In the marine environment, the dinoflagellates *Pyrodinium bahamense*, *Gymnodinium catenatum*, and several *Alexandrium* species, have the ability to produce PSTs (e.g. Oshima et al., 1993; Usup et al., 2012). The toxin profile in marine dinoflagellates is believed to be inherited in a Mendelian manner, and is thus constant among strains of the same species (Sako et al., 1992). However, the cellular toxin content is less stable phenotypically and varies among strains of the same species (Alpermann et al., 2010). The toxin production of Malaysian strains of *Alexandrium minutum* has been characterized in laboratory cultures (Usup et al., 2004; Lim and Ogata, 2005; Lim et al., 2006, 2010). These *A. minutum* strains produce mainly mono-sulfated GTX1/4 (>90% mole), with small amounts of GTX2/3 and trace amounts of STX and NEO (Lim and Ogata, 2005; Lim et al., 2006, 2007b). In accordance with other studies, the relative toxin composition of Malaysian *A. minutum* strains is relatively stable, predominantly constituted of GTX1/4, even under different N:P supply ratios, or under different N-nutrition conditions (Lim et al.,

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