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Influence of inorganic nutrition on growth and PSP toxin production of *Alexandrium minutum* (Dinophyceae) from Cork Harbour, Ireland

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Abstract

The physiological response of the PSP toxin producing dinoflagellate *Alexandrium minutum* isolated from the Irish coast was assessed after modulating the initial concentrations of nitrate and phosphate in batch cultures. The cell growth in cultures of strain CK.A02 was primarily controlled by nitrate availability. In all experiments, only gonyautoxins 2 and 3 (GTX2 and 3) were synthesized along the different growth phases, with GTX3 dominating ($\approx$80%) at all stages, making the GTX2–3 toxin profile a possible population marker of *A. minutum* in Cork Harbour. The cellular toxin quotas remained low and relatively stable at around 2 pg cell^{-1}, except when high N:P ratios were initially used for culture inoculations; in these conditions PSP toxins accumulated up to 14 pg cell^{-1}. Due to the composition of the toxin profile, the toxicity of strain CK.A02 was generally relatively low (from 1.1 to 1.7 pg STX eq cell^{-1}) in comparison with strains from other geographic areas except when phosphate limiting culture conditions were applied (maximum of 12.5 pg STX eq cell^{-1}). Results showed that sufficient soluble protein quotas were necessary to observe the intra-cellular accumulation of PSP toxins in phosphate limiting conditions, highlighting also the requirement of adequate nitrogen supplies. The possible existence of localized toxicity hot spots in the field, linked to the accumulation of PSP toxins within *A. minutum* cells as a metabolic response to adverse environmental conditions, could potentially increase risks for shellfish farming operations.
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Keywords: *Alexandrium minutum*; Harmful algal blooms; Paralytic shellfish poisoning

1. Introduction

Over the past decades, harmful algal blooms (HABs) have become a significant global concern as a food safety, environmental and socio-economic issue (Hallegraeff, 1993). Injurious algal species involved have had deleterious effects on water quality and aquaculture, and have sometimes led to severe human intoxications through the consumption of contaminated shellfish (Adams et al., 1968; Hoagland et al., 2002). Some species among the marine dinoflagellate genus *Alexandrium* are recognized as being responsible for paralytic shellfish poisoning (PSP). The highly potent neurotoxic

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compounds responsible for PSP are saxitoxin (STX) derivatives which may cause neurological symptoms, muscular paralysis and, in extreme cases, death in mammals (Kao, 1993; Shimizu, 1996). Amongst the *Alexandrium* genus, the potentially toxic bloom-forming species *Alexandrium minutum* occurs regularly in Western Europe, sometimes reaching high enough cell densities to cause water discolouration (Hansen et al., 2003; Garces et al., 2004). Blooms of the species generally arise in retention areas within harbours or estuaries, where they seem to be associated with a relatively stable water column and with freshwater inputs (Giacobbe et al., 1996; Vila et al., 2001). In countries such as Spain, Italy, Norway, Portugal, UK or France, significant impacts on utilization of shellfish resources happened where toxic *A. minutum* blooms have occurred (FAO, 2004).

Several studies have documented that the toxicity of *A. minutum* strains isolated from different regions around the world varies significantly. The subsequent determination of the toxin pattern of cultured isolates has often corresponded to the profile of contaminated shellfish in affected sites (Cembella et al., 1994; Chang et al., 1997). Not only does the toxicity fluctuate between locally or globally distributed isolates of *Alexandrium* species, but the toxin concentration in single isolates can change under different environmental conditions (Cembella, 1998). This variability in the overall toxicity is essentially attributable to modifications in cellular toxin amounts during the cell growth and transitional stages in the lifecycle of the organism but could also reflect changes in frequencies of individual toxin variant (Oshima et al., 1992; Alvito et al., 1995; Taroncher-Oldenburg et al., 1997; Hwang and Lu, 2000). The effects of a number of environmental and nutritional parameters on the physiology and toxicity of *Alexandrium* have been studied (White, 1978; Parkhill and Cembella, 1999; Hamasaki et al., 2001; Grzebyk et al., 2003). Nitrogen limitations have been reported to hamper both cell growth and toxin production (Chang and McClean, 1997; Wang and Hsieh, 2002). The influence of phosphate deprivation is, however, not as clear. Although phosphate limitation negatively affects cell division, increases of cellular toxin contents have been described in such conditions (John and Flynn, 2000; Lippemeier et al., 2003; Frangopulos et al., 2004). The effects of variations of initial nutrient concentrations on several *Alexandrium* species have been investigated but results remain difficult to compare because of differences in the experimental procedures. The importance of variability between strains is also poorly documented.

In Ireland, PSP toxicity in bivalve shellfish has been associated with *Alexandrium* blooms. These have resulted in the temporary closure of shellfish production sites in the North Channel of Cork Harbour, an estuary located on the south coast of the country. Recent investigations have shown that *A. tamarense* and *A. minutum* populations co-occur here (Touzet et al., 2006). *A. minutum* was identified as the organism responsible for the PSP events recorded in this region as (a) PSP toxin profiles obtained from cultures derived from locally *A. minutum* isolates coincided with those obtained from contaminated shellfish samples taken in 1996 (Furey et al., 1998; Touzet et al., 2006), and (b) *A. tamarense* isolates have proved non-toxic (Higman et al., 2001). Quantitative and/or qualitative toxin changes in cellular quotas must be considered as they can affect the overall toxicity in shellfish. The present study was undertaken to investigate the physiological effects of nitrate and phosphate variations on a toxic strain of *A. minutum* isolated from the North Channel of Cork Harbour. The development of the PSP toxin profile and amounts within cells was monitored along with the cell concentration, the cell size distribution and cellular soluble protein quotas in batch cultures initiated with a range of nitrate–phosphate conditions.

2. Material and methods

2.1. Culture conditions

The PSP toxin producing *A. minutum* strain CK.A02 was derived from a surface sediment sample taken in the North Channel of Cork Harbour after excystment of a resting cyst and subsequent isolation of a vegetative cell to generate a mono-clonal culture. Stock cultures were maintained in natural seawater supplemented with f/2 medium minus silicate (Guillard, 1975). Prior to the start of the experiments, a 1 l culture was grown to its late exponential/early stationary phase at a concentration of about 25×10^3 cell ml^{-1} for use as inocula (5% final volume). All media used thereafter were made up from natural seawater collected on the west coast of Ireland and filtered through a GF/C filter to remove coarse material. The seawater was kept in darkness for two weeks and filtrated through a 0.22 μm polycarbonate membrane.

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