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Temperature tolerance and expression of heat shock protein 70 in the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae)

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ABSTRACT

The aim of this study was to investigate the relationship between temperature tolerance and geographical origin in a species. Temperature tolerance and heat shock protein 70 (Hsp70) content were investigated in the toxic dinoflagellate *Alexandrium tamarense*, which was isolated from temperate and tropical areas. Results of treatment at 0–37 °C for 1 h revealed that 15 °C-cultured Japanese *A. tamarense* could survive treatment at 0–25 °C, whereas 30 and 37 °C treatment reduced the survival rate. Malaysian *A. tamarense* cultured at 25 °C survived at 30 °C; however, 37 °C and low temperature treatment reduced the survival rate. After acclimation of both strains at 20 °C, they were treated at 0, 4, 30, and 37 °C. The survival rate of Japanese *A. tamarense* at 30 °C was slightly increased compared to that of 15 °C-cultured cells. Treatment at 37 °C for 1 h showed no difference between acclimated and unacclimated cells of both strains. At 0 and 4 °C treatment, almost all cells of Japanese *A. tamarense* survived; however, the Malaysian cells were unable to survive. Both strains of *A. tamarense*, acclimated at 20 °C, were treated at 30 °C; change in the amount of Hsp70 was analyzed. Western blot analysis revealed that the induction of Hsp70 in the Japanese strain occurred more quickly than in the Malaysian strain. These results indicate that Hsp70 of *A. tamarense* is a heat stress-inducible protein and the response is different between strains.

1. Introduction

The dinoflagellate *Alexandrium tamarense* (Lebour) Balech is a marine phytoplankton that causes paralytic shellfish poisoning (PSP). PSP toxification of bivalves is a serious problem for fisheries and in food hygiene. Since *A. tamarense* is a harmful species and its toxicity and growth characteristics can be important information to work out a scheme for PSP, studies have been conducted to examine the relationship between environmental conditions and toxin production. Hamasaki et al. (2001) reported variability in toxicity of *A. tamarense* based on environmental conditions. They showed that cell toxicity in *A. tamarense* isolated from Hiroshima Bay increased in low salinity environments with high ammonium concentrations and low light intensity. Temperature is considered to be an important factor for occurrence of blooms and toxin production. Ogata et al. (1987) reported that the growth rate of *A. tamarense* declined and the amount of cellular toxin rose in response to a drop in water temperature. This finding is consistent with previous observations that the toxicity of natural cells of the temperate strain of *A. tamarense* at lower temperatures is greater than those in higher temperature waters (Ogata et al., 1982). In Ofunato Bay (North eastern Japan), *A. tamarense* appears within the temperature range of 5–14 °C (Fukuyo, 1982). On the other hand, in tropical areas, seawater temperature is consistently above 20 °C, and Malaysian strains have been maintained at 25 °C (Lim and Ogata, 2005). These Malaysian strains were morphologically identified as *A. tamarense*, although the nucleotide sequence of the nuclear ribosomal RNA gene was similar to that of *A. affine* (Leaw et al., 2005). *A. tamarense* has been found in various areas of the world, such as South America, South Africa, Australia, the Pacific Islands, India, Asia, and the Mediterranean (Lilly et al., 2007), thus indicating that *A. tamarense* can survive in a wide range of water temperatures. Although some characteristics of the relationship between temperature and growth in *A. tamarense* have been identified (Watras et al., 1982), the mechanism underlying the response to change in water temperature has not been examined.

Heat shock protein 70 (Hsp70) is an important molecule associated with heat stress response. It plays an important role in molecular chaperone and protein turnover during translation and maturation of proteins. Hsp70 prevents aggregation of denatured proteins and refolding of proteins denatured by heat stress (Sonna et al., 2002). It has a highly conserved sequence across all organismal kingdoms, and its expression is induced by heat stress

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